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Proceedings of Faculty of Medicine Academic Session (FMAS) – 2019, University of Ruhuna

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Cover Story

Achievements of Ethics Review Committee Faculty of Medicine, University of Ruhuna in 2017 - 2018

Ethical Review Committee (ERC), Faculty of Medicine University of Ruhuna was established in 1985.

The present ERC took office in March 2017 with the leadership of Prof. A de S Nagahawatte and expedited application for “Strategic Initiative for Developing Capacity in Ethical Review” (SIDCER) recognition.

Prof. Saman Wimalasundera, former Dean, provided a permanent place for ERC office. Membership of the Forum for Ethics Review Committees in Sri Lanka (FERCSL) was obtained in July 2017.

In 2018, ERC was offered international recognition under the SIDCER of the Forum for Ethical Review Committees of Asia and the Western Pacific (FERCAP). Dr Chandima Madhu Wickramatilake, Secretary, ERC, attended the FERCAP conference and awarding ceremony held in Taiwan. FERCP is a network of independently established regional fora for ethics review committees, health researchers and invited partner organizations involved in Research Ethics.



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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), Key- words, 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.



Message from the Chairperson

I am privileged to release this message on behalf of the organizing committee of the Faculty of Medicine Academic Sessions (FMAS)-2019, Faculty of Medicine, University of Ruhuna. I am delighted to welcome all of you to the 7th FMAS, the pinnacle of annual calendar of faculty events. It is the time not only for sharing knowledge, reviewing and showcasing the scientific output from the Faculty but also it is the time for gathering and networking.

The theme of this year's academic sessions is “Addressing the contemporary nutritional challenges in Sri Lanka” which focuses on current trends in nutritional disorders prevailing in Sri Lanka and their management. It is worthwhile to note that this year's academic sessions is enriched with keynote address, the oration, the symposium, presentations on completed postgraduate's degrees and two free paper sessions (open category and the undergraduate category).

Symposium is conducted by an eminent faculty especially selected to talk on the nutrition related fields. In particular the target audience is medical undergraduates, the budding doctors serving the country where the burden of non-communicable diseases is increasing alarmingly. The symposium lecture topics are useful to everybody not only to gain scientific knowledge, but also to improve the personal health. During the inauguration the students with best overall performances in the faculty are presented with Dean's Awards which encourages the students to perform better both in academic and extracurricular activities.

I am extremely grateful to the FMAS organizing committee for their unstinted support to make it a reality and I wish to convey my sincere gratitude to the all the resource persons, orator and the presenters for their contributions to make this event a success.

Dr. Chandima Madhu Wickramatilake
Chairperson, FMAS-2019



Message from the Vice Chancellor

In the first instance let me congratulate the Dean and the organizing committee chaired by Dr. CM Wickramathilake for successful organization of the FMAS 2019. The theme “Nutritional challenges of the 21st century “ is very timely as Sri Lanka is facing a major calamity called non-communicable diseases in which nutrition plays a huge role in aetiology.

Diabetes, hypertension and ischaemic heart diseases are causing huge losses in productive lives of relatively young people in Sri Lanka and in addition to economic losses. Behavioral changes starting from young children and adolescents will change this picture over the next 20 -30 years. It is a long term problem with no immediate solution.

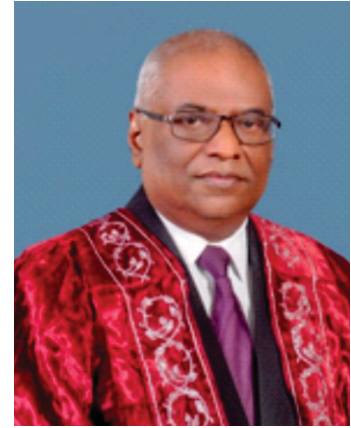
Focus on life cycle approach to start changing behaviors at crucial age groups like under 5 children, school children, young persons from 15 to 25 years and adults between 25 to 50 /60 years is necessary to achieve these objectives.

The programmes required to face nutritional challenges of this century for each age group are different. There are enough evidence-based approaches and programmes that could be implemented or adopted immediately into our country and the health system to achieve this. We just need to do it rather than talk about it. Can we be pragmatic and practical in our mindset to face these challenges will remain as our question?

FMAS while promoting research in this area and providing a platform for presentation of research in this area should focus the discussions towards changing the mindset of academic staff who are in policy making positions to reach grass roots and do it to achieve these targets.

I wish FMAS 2019 all the best.

Senior Professor Sujeewa Amarasena
MBBS(Ruh)MD(Paed- Col), DCH (Col),DCH(Sydney), FSLCPaed
Vice Chancellor
University of Ruhuna



Message from the Dean

I am privileged as the Dean of the Faculty of Medicine, University of Ruhuna to send this brief message to Faculty of Medicine Annual Academic Sessions (FMAS) August 2019. I congratulate the Chairperson and the organizing committee for making this important event a reality while appreciating their continuous and tireless effort on this project over last few months. The success of this event itself is an evidence to show the strength of the Faculty of Medicine of the University of Ruhuna and its commitment to maintain best practices as an academic institution.

Considering the caliber of the resource persons who contribute in the academic sessions, I am sure that it will be a fruitful academic experience on multiple facets of nutrition for all who attend the sessions including the medical students. While providing a forum for the Faculty to share their research experiences with colleagues, FMAS gives an opportunity for the medical students to present their research findings and this practice will contribute to the future development of medical sciences in the country.

I would like to express my gratitude to all the members in the FMAS team who worked hard to make this event a success and I wish them all the best.

Professor Vasantha Devasiri
MBBS (Ruh), DCH (Col) MD (Col) FSLCP
Dean, Faculty of Medicine
University of Ruhuna

Inauguration Ceremony of FMAS-2019

Thursday 8th August 2019

T.W. Wickramanayake Auditorium, Faculty of Medicine, University of Ruhuna

1700 hrs	Guests to be seated
1710 hrs	Ceremonial procession
1715 hrs	Faculty & University songs
1720 hrs	Lighting of the oil lamp
1725 hrs	Welcome speech Dr. Chandima Madhu Wickramatilake Chairperson, FMAS-2019
1730 hrs	Address by the Dean, Faculty of Medicine, University of Ruhuna Professor Vasantha Devasiri
1740 hrs	Address by the Chief Guest, Vice Chancellor, University of Ruhuna Senior Professor Sujeewa Amarasena
1750 hrs	Keynote Address 'Achieving Life style modification - How practical is it?' Dr. Renuka Jayatissa Head of the Department of Nutrition, Medical Research Institute Visiting Consultant Medical Nutritionist, National Hospital of Sri Lanka
1840 hrs	Cultural performance
1850 hrs	Dean's Award 2019
1905 hrs	FMAS Oration-2019 Impact of salt iodization in Sri Lanka on iodine status during pregnancy and neonatal thyroid functions: A rational approach for policy makers Dr. Eric De Zoysa Senior Lecturer in Biochemistry, Department of Biochemistry, Faculty of Medicine, University of Ruhuna
1955 hrs	Vote of thanks Dr. Nayani Prasangika Weerasinghe Secretary, FMAS-2019
2000 hrs	Reception

Faculty of Medicine Academic Sessions (FMAS) - 2019
University of Ruhuna

Friday 9th August 2019

T.W. Wickramanayake Auditorium, Faculty of Medicine, University of Ruhuna

0800 hrs	Registration
0830 - 1000 hrs	Presentations based on completed higher degrees Chaired by Professor K.D. Pathirana and Professor Isurani Illeyperuma Dr. Harshani Thabrew, Department of Microbiology Faculty of Medicine, University of Ruhuna Dr. Thyagi Ponnampereuma, Department of Community Medicine Faculty of Medicine, University of Ruhuna Dr. Pabasara Kalansuriya, Department of Biochemistry Faculty of Medicine, University of Ruhuna
1000 - 1030 hrs	Tea & Poster session
1030 - 1230 hrs	Symposium
1030 - 1055 hrs	‘Current trends in nutritional disorders in Sri Lanka’
1055 - 1120 hrs	Dr. Ranil Jayawardena Senior Lecturer, Department of Physiology, Faculty of Medicine, University of Colombo, Sri Lanka
1120 - 1145 hrs	Dr. Thejana Wijerathne Senior Lecturer, Department of Surgery, University of Sri Jayewardenepura, Sri Lanka
1145 - 1210 hrs	Dr. Banuja Wijayathilaka Consultant Community Physician of Nutrition Division Ministry of Health, Nutrition and Indigenous Medicine, Sri Lanka
1210 - 1230 hrs	Panel Discussion
1230 - 1315 hrs	Lunch Free paper sessions
1330 - 1430 hrs	Open category Chaired by Prof. Menik Hettihewa and Prof. Manjula Hettiarachchi
1435 - 1605 hrs	Undergraduate category Chaired by Prof. Channa Yahathugoda and Prof. Sudheera Jayasinghe
1605 - 1620 hrs	Awards Ceremony
1620 - 1635 hrs	Tea

Achieving Life style modification - How practical is it?

Dr. Renuka Jayatissa (MBBS, M.Sc, MD)
President Sri Lanka Medical Nutrition Association
Head of the Department of Nutrition Medical Research Institute



Sri Lanka is facing the epidemic of non-communicable diseases due to adaptation of wrong life style. Therefore adopting the correct life style by modifying it throughout the life cycle is the foundation to tackle this epidemic.

Lifestyle modification includes 3 primary components: diet, exercise, and behaviour therapy. Adopting the diet is the vast subject, which need individual approach and it should be supported by public health interventions. There are many fashionable diet regimes available such as intermittent fasting, keto diet, Atkins diet, no or low carb diet, skipping meals etc. Calorie restriction rather than changing macronutrient composition of the diet, is the key determinant of weight loss.

Because all of the diets reviewed appear to have comparable short- and long-term safety, the choice of the diet can be guided by the desired control of comorbid conditions. Overweight and obese individuals, as well as persons of average weight, often report not having time to exercise. Medical practitioners should emphasize on moderate intensity activities, even in short bouts, as brief as 10 minutes. A comprehensive program of lifestyle modification produces a 7% to 10% reduction in initial weight that is associated with clinically meaningful improvements in risk factors of Cardio Vascular Diseases, including the prevention of type 2 diabetes. Internet based life style modification programmes are new developments, which provide the full knowledge to modify life style within the home settings rather than visiting gymnasiums. These initiatives must be complemented by public health efforts to prevent non communicable diseases. Life style modification should be a part of everybody's life at all stages with specific attention to pre-schools, schools, universities, work sites and elderly life.

Healthy eating; is it happening?

Dr. Bhanuja S. Wijayatilaka (MBBS, MSc, MD)
Consultant Community Physician of Nutrition Division
Ministry of Health, Nutrition and Indigenous Medicine, Sri Lanka



The concept of “healthy eating” is something that many of us are familiar with. However, the perceived ideas are so diverse and could be incomplete though each may have some elements relevant to the concept. Nevertheless, looking for a comprehensive definition is not an aim in this work but understanding some salient facts and gaps of healthy eating in Sri Lankan context. Healthy eating should be enjoyable as eating is one of the joys of life. It is not about strict dietary limitations or depriving yourself of the foods you love because healthy eating does not depend on the food alone. It's actually related to the habit of food consumption, amount and frequency depending on the physiological status of the person. Thus, the real problem is that many are not used to being satisfied with the correct amounts of food. Hence, healthy eating indeed is a skill of developing to be happy, feeling great and boosting your mood with the 'healthy' amount of food. Therefore healthy eating is really a challenge and a skill to be developed from the beginning of life.

It is hard to claim for a completely healthy food as each food could have some important element missing. However, healthier food options for Sri Lankans have been described in Food Based Dietary Guidelines developed by the Ministry of Health. In the meantime, considering the current trends of overweight/obesity and diet related non-communicable diseases, a scientific tool, the Nutrient Profile Model for Sri Lanka has been developed to identify food with higher amount of components such as fat, salt, sugar and energy which have been proven that if consumed beyond the limit as determinants for such conditions. Nutrient Profile Model is a globally accepted scientific method to identify food that are more likely to be constituents of a healthy diet and could be used for many public health interventions.

Several concerns in healthy eating have been identified in Sri Lankan context. Consumption of big main meals particularly high in starch and fat (irrespective of homemade or commercially prepared) and gradual disappearance of vegetables and green leaves from the food plate are some main issues in Sri Lankan diet. Consumption of many snacks, probably high in fat, salt, sugar and energy such as deep fried short eats, biscuits, bakery products and sweetened beverages is another serious problem leading to diet related non-communicable diseases.

Adding of high amounts of oil, salt and sugar in our traditional meal preparations, lack of awareness on hidden salt and sugar in ready to eat food items, poor consumption of healthy fat containing food such as oily nuts and some fruits as well as frequent exposure to food prepared using used oil are some other problems that would have contributed to increased trends in diet related non-communicable diseases. Insufficient intake of food with high quality proteins also have been identified as a problem in our community. Hence, consumption of low cost but high quality protein containing food such as eggs and small fish should be promoted as a part of healthy eating. Iron deficiency is still a problem specially among children and pregnant mothers. Inadequate consumption of locally available iron rich food including food of animal origin is another issue in healthy eating. Average fruit consumption among our population is also low when compared with the recommendations.

In this scenario, making public awareness through guidelines and other means is an essential element in developing healthier food habits. Non availability of healthier food options including healthy snacks has made serious negative impact on healthy eating. Hence, making healthier food options available and affordable for the general public through creating a supportive food environment would be the most powerful element to establish healthy food habits in our community. However, developing a generation who loves the taste of natural fruits and vegetables while preventing them from getting addicted to the flavor of high fat, salt and sugar would be the strongest factor to make healthy eating sustainable, because it's about nothing but developing the skill of being happy, feeling great and boosting the mood with healthier food options.

Plate model concept

Dr. Ranil Jayawardena (MBBS, MSc, PhD)
Registered Nutritionist
Senior Lecturer, Faculty of Medicine, University of Colombo



Dietary patterns in Sri Lanka are rapidly changing with urbanization. A study shows a considerable proportion of adults failing to follow recommended dietary guidelines. Nearly 70% exceeded the recommendations for starch (carbohydrate) intake, while the daily intake of fruit and vegetable portions dropped well below national recommendations, with only 3.5% of adults consuming the recommended five portions of fruits and vegetables per day.

Excess carbs consumption seems to have become the main issue of people. Although a heaped-up plate of rice was fine for those who toiled the land but not now for those who are seated (sedentary) most of the time. Another worrisome matter is that Sri Lankans do not seem to be eating enough protein.

Sri Lankans obtain nearly 72% of their calorie requirement through carbohydrates (mainly refined), while only 10% of calories are derived from proteins. In a typical Sri Lankan rice plate, there is about 400g of rice and very little vegetables and protein. Thus, cutting down the amount of rice from each meal will help reduce excess calories from the main meals and lead to body weight reduction eventually. With an increase in vegetables and protein-rich foods to achieve daily targets, there will be other health benefits and maintenance of lean mass.

Obesity is defined as “deposition of excess body fat” and according to the World Health Organization (WHO), people with body mass indexes (BMIs) of 25.0 kg/m² to 29.9 kg/m² are considered as overweight and BMIs of 30.0 kg/m² and above are labelled as obese. However, since Asians tend to have higher amounts of body fat at lower BMIs and a higher risk for obesity associated diseases such as diabetes and cardiovascular diseases, these cutoffs may be insufficient in identifying South Asians individuals with a high risk of obesity-related morbidity and mortality.

Losing weight is all about 'Calories In vs. Calories Out'. To lose weight, you need to consume a few less calories each day than your body burns. In the energy deficit method, you have to measure your calorie intake and consume appropriate amount. However, measuring calories in Sri Lankan foods is not possible practically because there is no complete composition database for Sri Lankan mixed dishes. Secondly, there is a considerable variation in the recipes of the local curries.

'My Rice Plate' which is culturally specific has been designed according to the 'Plate Model' recommended by local and international nutrition guidelines. Ongoing clinical trials have revealed that 'My Rice Plate' causes a considerable reduction in body weight and waist circumference and an improvement in plasma blood glucose levels in obese adults (BMI>25kg/m²).

Feasibility and outcomes of bariatric and metabolic surgery in Sri Lanka Experience of a single center over 10 years

Dr Thejana Kamil Wijeratne(MBBS, MS, MRCS)
Senior Lecturer in Surgery, Department of Surgery,
University of Sri Jayewardenepura, Sri Lanka



Surgery for extreme obesity and control of metabolic diseases has been introduced to Sri Lanka relatively later than our neighboring countries. Although well accepted and practiced in other countries in Asia these procedures have not yet gained the adequate recognition and acceptance by the medical professionals in Sri Lanka where Morbid Obesity and Metabolic syndrome increases rapidly leading to many health and social issues among all sectors of our population.

Laparoscopic Bariatric and later on the metabolic surgical procedures have been initiated at Colombo South Teaching Hospital 10 years ago and today has been developed into a high volume obesity management center serving referrals from around the country. The effectiveness and outcomes of these procedures have been studied prospectively with regular follow up of patients and a updated database ; therefore enabling us today to confidently discuss the feasibility and outcomes of these procedures among Sri Lankans.

The three most commonly used Bariatric Surgical procedures in the world namely the Laparoscopic Sleeve Gastrectomy (LSG), Laparoscopic Mini Gastric Bypass (MGB) and Laparoscopic Roux on Y gastric bypass have been performed on selected patients to obtain the desired weight and co-morbidity management.

Overall weight loss of > 30% have been achieved by majority in first year and 90% of patients managed to reach normal BMI within 18 months post surgery. Complete resolution of diabetes and hypertension have been achieved by over 80% and in the remaining good control of disease achieved. Fatty liver reduction was achieved at a rate of one radiological grade per year. All females who underwent weight related subfertility managed to conceive within 2 years. All patients with Obesity Hypo-Ventilation syndrome (Obstructive sleep apnoea) managed to discontinue their home C-PAP machines within 6 months post surgery.

After completing 100 successful procedures the unit managed to perform surgery on patients with extreme weight (Over 200 kg), Children (Youngest 13 years) and including children with syndromic obesity. Several adults with known psychological disorders and learning difficulties were also managed successfully.

Laparoscopic Bariatric and metabolic Surgery can be successfully performed in Sri Lanka with excellent outcomes with acceptable mortality and morbidity rates.

Current trends in childhood nutritional disorders in Sri Lanka

Pujitha Wickramasinghe (MBBS (Col), MD (Paed), DCH (Col), PhD (Col)
Senior Professor in Paediatrics, Department of Paediatrics
University of Colombo



Nutrition is one of the key factors that would determine health of an individual. Effects of childhood nutrition are long term, where it directly contributes to the onset of non-communicable diseases later in life. Changes in the economy have lead to changes in feeding behavior resulting in changes in the demography of nutritional disorders.

Closed economic policies with priority in domestic production lead to have low purchasing power and low availability of food in the country in mid part of twentieth century, where stunting, wasting, iron and iodine deficiency were the main nutritional disorders in the country. Changes in economic policies provided more purchasing power and availability of food helped to combat old nutritional disorders but paved the way to the emergence of new problems.

The nutritional status of the children under 5 years of age from 1975 onwards have reduced gradually. Underweight and stunting have reduced from almost fifty percent to less than twenty five percent in little over a quarter of a century. However, wasting had been remaining almost the same around 13-14% throughout this period. Since the turn of the century, wasting and stunting plateaued.

Data on 5-10 year old children has shown that from the beginning of this century, there is a slight increase in wasting while there is marked reduction in stunting rates. However, the obesity and overweight rates are increasing gradually and in and around major cities it is about 10 -15%.

The landscape of micronutrient status of the country is also changing. Most of the data were based on indirect assessments and very recently only micronutrients were directly assessed. Vitamin A which was a public health problem at the turn of the century in under 5 year old children, has been successfully controlled with the introduction of vitamin A mega dose supplementation. Use of iodine fortified salt has brought successful results. However, iron deficiency has not been successfully controlled although iron deficiency anaemia in children is not a major health problem today. New emerging areas that need attention are zinc deficiency and also vitamin D deficiency.

Despite improvement in the health status of the country, the undernutrition indicators have become static. Why we have not been able to improve them further? Are we using wrong indicators? Are we over diagnosing stunting and under diagnosing overweight, obesity? These are some of the questions that has been discussed at the scientific, managerial and policy formulating circles. Could we find an answer to all of these?

Trauma Exposure and Post-trauma Mental Health Problems: Risk and Resilience in Adolescents

Thyagi Ponnampereuma

Department of Community Medicine, Faculty of Medicine, University of Ruhuna

Abstract

Each year, millions of children around the globe are exposed to trauma. Although it is well established that exposure to trauma leads to a wide range of mental health problems, variation in the prevalence and severity of these psychopathologies cannot be fully explained by the type of trauma and by the severity of the traumatic stress itself. Present study therefore investigated several risk and resilience factors that influence the trauma – mental health pathway. Initially (T1), a school-based screening was conducted in a sample of 753 adolescents aged 13-16 years, in an area impacted by the 2004 tsunami. Participants completed measures of trauma exposure, negative cognitive appraisal, daily stressors, social support, PTSD symptoms, emotional and behavioral problems (EBP), and functional impairment. A follow up study (T2) was done 16 months later in a subsample of 90 adolescents. All questionnaire assessments were repeated and saliva samples were collected 4 times a day for 3 days to measure bodily cortisol level. In the 60% of the T1 sample reporting any trauma exposure, 24% met criteria for full or partial PTSD. EBP were reported in 13% of adolescents. Based on the results, negative appraisal was the best predictor of posttraumatic stress disorder symptoms (variance 21.9%) and the relationship was specific to the disorder. Daily stressors appeared to be an important transmitter of the impact of trauma on adolescents' mental health problems. Further, the social support moderated direct and indirect pathways from trauma to daily impairment. Basal cortisol level, diurnal slope or the cortisol awakening response were not significantly associated with trauma exposure or with PTSD symptoms. But the results showed a robust gender difference where the trauma exposed girls with PTSD showed high cortisol level compared to similarly exposed boys. Adolescents with PTSD had increased level of EBP and PTSD symptoms together with EBP heighten the risk for impaired daily functioning. In closing study emphasizes that the trauma exposure is common among adolescents.

Adolescents with PTSD symptoms can especially benefit from cognitive interventions. Further, minimizing daily stressors will improve post-trauma mental health, in particular among adolescents with low social support. The observed gender difference in cortisol level may support the notion of gender-specific increases in the sensitivity of the HPA system to trauma during mid-adolescence, which may explain the female vulnerability to PTSD. Although findings supported the available evidences on risk and resilience factors for post trauma mental health problems, longitudinal studies are needed to confirm the causal relationships of all these associations.

Introduction

The incidence of traumatic events has increased in the world, exposing many children and adolescents to the burden of psychological trauma. Epidemiological studies point out that 60% of adolescents experience at least one potentially traumatic event by the time they reach adulthood (1-3). These traumatic experiences contribute to many different psychological problems such as posttraumatic stress disorder (PTSD), depression, anxiety and other emotional and behavioral problems related to life functioning. PTSD is the most common, trauma specific psychopathology in victims and it is reported in about 16% of trauma exposed adolescents (4). General psychological problems such as emotional and behavioral problems (EBP) were also frequently reported in traumatized adolescent and the reported prevalence was 23% (5-6). Many adolescents with these post-trauma psychological problems recover within few months, but around 5% of the victims continue to have debilitating chronic symptoms for many more years in life. Better understanding of the vulnerability for post-trauma psychopathologies in this age group is therefore important in facilitating clinicians and public health teams to target prevention and clinical interventions.

The risk and resilience factors identified are broadly categorized as trauma related factors and individual factors. Trauma-related pathological factors of importance include the degree, type, and duration of trauma exposure and cumulative trauma exposure. Individual factors are again classified as psychological factors as such as co-occurring psychological problems, maladaptive coping strategies and maladaptive cognitive appraisal of trauma, social factors such as unfavorable social environments (i.e., low social support) and daily stressors (7). Biological factors such as female gender, young age, genetic polymorphisms and endocrine function alterations (8). Although many studies have been carried out to identify possible risk factors post-trauma psychopathologies, many gaps remain in the literature (9-10). This study therefore attempts to expand the existing knowledge on several vulnerability factors: negative cognitive appraisal as a psychological risk factor, daily stressors as a social risk factor and bodily cortisol level as a biological factor in adolescence (11-12). In addition the study examined the co-occurrence of EBP with PTSD in traumatized adolescents (13).

The negative cognitive appraisal of trauma and/or its sequelae have been associated with PTSD. According to the cognitive model formulated by Ehlers and Clark (2000), PTSD symptoms arise and are maintained when an individual processes the traumatic event and subsequent symptoms in a way that leads to a sense of current threat, and subsequent anxiety. Thus, a traumatized individual would interpret a traumatic experience and subsequent symptoms as evidence of personal weakness or permanent damage, thereby experiencing the world as a dangerous place. To obtain temporary relief of these symptoms, individuals develop maladaptive behaviors and cognitive strategies. However, these adaptations impede correcting negative appraisals and memories, thereby resulting in persistent symptoms of PTSD long after the traumatic event. Many studies in adults have provided evidence that negative appraisal affects PTSD, but studies in children or adolescents have lagged behind.

Daily stressors are the other important risk factor in post-trauma psychological problems. Daily stressors are the environmental events or chronic conditions that objectively threaten the physical and/or

psychological well-being of individuals (14). In the aftermath of traumatic events, daily stressors were found to be increased and were associated with mental health problems (15-17). Therefore, it is plausible that daily stressors may influence the trauma-mental health relationships but the mechanism of its effect is not clearly understood. The mediation hypothesis of daily stressors in trauma mental health pathway was supported mainly by studies conducted in adult survivors, but studies in young victims are limited. Further, previous studies have also demonstrated that social support buffers the impact of trauma and subsequent life stressors on developing PTSS, but no study has investigated the possibility that social support might moderate the indirect pathway from trauma to mental health outcomes, via daily stressors (18-19).

Hypothalamic-pituitary-adrenal (HPA) axis dysregulation in trauma exposure is also investigated to understand the pathophysiology of PTSD (20). Trauma studies in adults have generally revealed lower cortisol basal levels in individuals with PTSD, but studies in adolescents with PTSD have shown inconclusive results. Lack of clear consensus with respect to the young age group is possibly because cortisol secretion depends on many factors such as age, gender, developmental stage, body mass index, trauma characteristics, daily stressors etc. Gender effects on HPA response to trauma in adolescents are also important, as gender differences might theoretically help to explain heightened female vulnerability to PTSD (21). Studies investigating diurnal cortisol secretion in adolescents, and gender differences in these associations have less investigated in trauma psychology studies.

A high prevalence of comorbid psychiatric disorders have been noted in adolescents with PTSD. Recent studies have demonstrated that PTSD increased the risk of other psychopathologies following trauma (22). Several studies have investigated this link between PTSD and co-occurring major psychiatric disorders such as depression McMillen et al. (2002), but the association of PTSD to common general psychological problems such as emotional and behavioral problems (EBP) of adolescents has drawn less attention (23). Further, relatively little research has been conducted on how psychological problems impact daily functioning and, more specifically, how

comorbid psychopathology adds to the problem of impairment.

To fill the above mentioned gaps in knowledge this study addressed the following research questions.

- How do personal factors such as cognitive appraisal and daily stressors influence PTSD and other mental health problems in trauma-exposed adolescents?
- Does negative cognitive appraisal of trauma predict posttraumatic stress symptoms? If so, what determines negative cognitive appraisal of trauma?
- Are daily stressors associated with PTSS and impaired functioning? If so, what is the role of daily stressors in these associations? Does social support influence the relationship?
- Is the biological stress system linked to trauma exposure, or does it reflect subsequent symptoms?
- How are basal cortisol level, cortisol diurnal slope, and cortisol awakening response associated with trauma exposure and PTSS in adolescent girls and boys?
- To what extent do emotional and behavioral problems coexist with PTSD symptoms in adolescents, and how does cumulative psychopathology impact daily life functioning?
- Do adolescents with PTSD experience more EBP relative to either the PTSD negative or the trauma-free group? If so, how do cumulative psychopathologies affect the daily life functioning?

Methods

This study was conducted among adolescents exposed to different types of trauma in Sri Lanka. As shown in Figure 1, the research was carried out in two phases. The initial school-based screening was done with 755 adolescents aged between 12-16 years. Two study locations were selected with different degrees of tsunami impact: Hikkaduwa area, in the coastal belt, with high tsunami impact, and Bope-Poddala, 6 km inland from the coast, with less impact. Government

assessments after the tsunami documented that 9 of the 40 secondary schools in Hikkaduwa but none of the 18 schools in Bope-Poddala area had been directly affected by the disaster. The study randomly selected five affected schools and five unaffected schools from the two areas, such that selected schools in each area were representative of the three categories of secondary education in the country. The study used a self-reported questionnaire to measure trauma exposures, psychological problems (PTSD, EBP, and functional impairments), risk factors, including negative cognitive appraisal and daily stressors, and one probable protective factor: social support.

The follow-up study was conducted 16 months later in a sub-sample of 90 adolescents selected from the baseline study, according to the presence or absence of trauma history and psychopathology. Saliva was collected to measure bodily cortisol level in a stress free manner. Same questionnaires were used to assess mental health problems, risk and resilience factors. The study was approved by the ethics committee of the Faculty of Medicine, University of Ruhuna, Ministry of Education and the school principals.

Measures

Lifetime traumatic experiences Adolescent version of UCLA PTSD Reaction Index for DSM-IV (PTSD-RI) was used to assess lifetime exposure to 13 categories of traumas (24). A cumulative trauma score was formulated as the number of different event categories a participant endorsed. Severity of trauma (Criterion A) was measured by 17 items.

Phase I Study -School- based screening of trauma exposure and mental health problems of adolescents

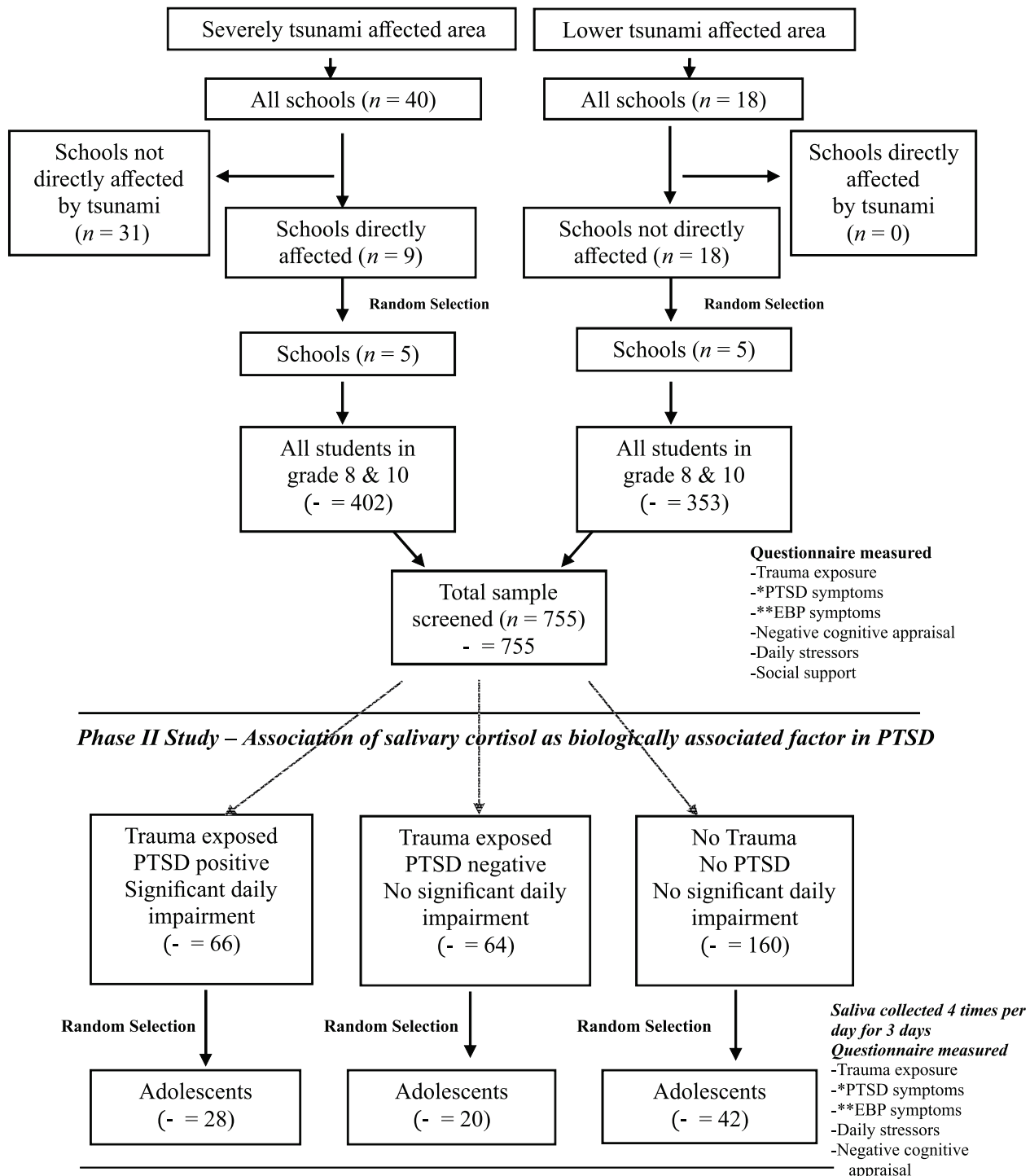


Figure 1. Study design

Note: *Post-Traumatic Stress Symptoms, ** Emotional and Behavioral Symptoms

Posttraumatic stress The PTSD-RI was also used to screen for PTSD symptoms and diagnostic criteria. The PTSD-RI has been validated and shown good psychometric properties in previous studies in Sri Lanka. The 22 items measures DSM- IV-TR symptom clusters: re-experiencing, avoidance, and hyperarousal. The total scores of these clusters provides a measure of PTSD symptom severity (PTSS) (Cronbach's $\alpha = .89$). PTSD-RI categorized individuals as having full PTSD, partial PTSD, no PTSD (Steinberg et al. 2004).

Emotional and behavioral problems (EBP) Participants completed the extended adolescent self-report version of the Strengths and Difficulties Questionnaire (SDQ) previously validated in Sri Lanka (25-26). High scores on this instrument have been associated with risk of mental disorders (27). Five subscales (emotional, conduct, hyperactivity and peer, prosocial problems) measure EBP (Cronbach's $\alpha = .66$). Emotional and peer problems measures internalizing problems. The established three-band norms categorized SDQ scores as normal, borderline, or abnormal.

Psychosocial impairment The impact supplement of the SDQ is a reliable measure of impairment related to psychological problems in adolescents (28). It assesses the extent to which reported EBP (a) upset or distress the respondent and (b) interfere with everyday life. The total score measure impairment (Cronbach's $\alpha = .73$).

Negative trauma appraisals This was assessed as the sum of ratings on 8 items derived from a 10-item trauma appraisal questionnaire developed for children (29). This is also not validated to Sri Lankan adolescents, but the questionnaire was translated according to stranded procedures and the scale demonstrated a good reliability (Cronbach's $\alpha = .79$).

Current daily stressors, Measured by the Long-term Difficulties Questionnaire for Youth (LDQ-Y). Adolescents indicated difficulties experienced in the following areas: school, home, the neighborhood, leisure activities, peer relations, family relations, health, worrying about the future. Even though this is not validated to Sri Lanka, it showed a good reliability (Cronbach's $\alpha = .79$).

Social support The Multidimensional Scale of

Perceived Social Support (Zimet, Dahlem, Zimet, & Farley, 1988) measures perceived support from friends, family, and a significant other ("special person"). Even though this scale was not validated specifically to Sri Lankan adolescents, it has been validated and has shown good psychometric properties in diverse ethnocultural context (Wilson, Yendork, & Somhlaba, 2017). The mean item score (5.09) was similar to that reported in previous studies and the reliability was Cronbach's $\alpha = .78$.

Salivary cortisol

Basal cortisol secretion was measured by obtaining 12 samples: on awakening, 30 min later, in the late afternoon, and before going to bed, on 3 consecutive days. They received polyester swabs (salivettes, Sarstedt, Germany) in a bottle with an electronic cap (MEMS6, Aardex, Switzerland) that registered opening times. Samples of 1055 tubes were frozen until shipment. After exclusion a total of 900 valid samples (average of 10.71 samples per person) were analyzed in the laboratory at TU Dresden, Germany (IBL, Hamburg, Germany).

Statistical Analysis

Analyses were performed with SPSS, with two-tailed p -values ≤ 0.05 considered significant. Missing values were imputed with the SPSS multiple imputation procedure before scale scores were calculated. ANCOVA with Post-hoc comparison, chi-square analysis with post-hoc comparison and hierarchical multiple regression analyses (entry method) were performed to identify the associations of possible risk and resilience factors to post trauma mental health problems (PTSD, EBP, impaired functioning). Multilevel regression analyses was performed to examine the association of basal cortisol level, diurnal slopes and cortisol awakening response to PTSD symptoms. All models were controlled for possible confounding factors. To test hypotheses concerning mediation and moderated mediation analysis, SPSS macro PROCESS, with bias-corrected bootstrap 95 % confidence intervals (1,000 samples) was performed. (30-31)

Results

Sample characteristics

The T1 sample comprised 753 adolescents (53.9% girls), aged 12 to 16 years (mean 13.60, SD = 1.11). Similar percentages of participants came from high (53.9%) and lower (46.1%) tsunami impact areas. A majority (60.3%) of the participants had been exposed to at least one category of traumatic event (figure 2); 41.4% reported multiple traumatic experiences, with a mean of 1.79 (SD = 1.98, range 0–10). Boys reported significantly more total trauma types than girls, with means of 1.86 vs. 1.54, respectively, $t(751) = 2.27$, $p = .023$. The T2 follow-up sample included 89 participants (57.3% girls), aged 13 to 18 (mean 14.39, SD = 1.10).

In the initial screening, PTSS ranged from 0–64, with a mean of 17.0 (SD 13.0). DSM-IV criteria for full or partial PTSD was met by 106 adolescents (14.0% of the total sample; 23.5% of the trauma-exposed subsample), of whom 36 (4.8%) with full PTSD and 70 (9.3%) with partial PTSD. Girls reported more severe symptoms of PTSD than boys $t(751) = 2.61$, $p = 0.009$. EBP scores ranged from 0–28, with a mean of 9.9 (SD = 5.1). Based on SDQ cut-offs, levels of EBP were abnormal in 37 (4.9%) of the 753 participants and borderline in 64 (8.5%). Over the entire sample 122 participants (16.7%) had SDQ impact scores in the borderline to abnormal range, indicating moderate to severe psychosocial impairment.

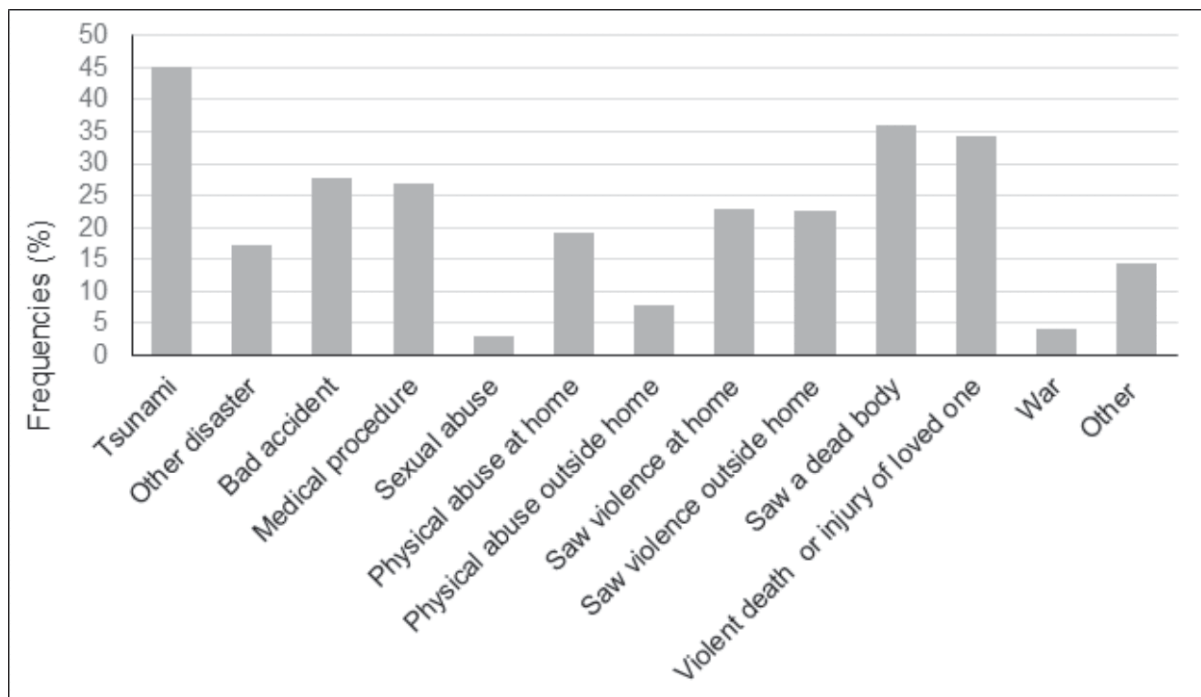


Figure 2 Traumatic event exposure by type

Association of Negative appraisal to PTSD symptoms

Negative appraisal was predicted by severity of trauma exposure abusive events, greater cumulative trauma, or greater current adversity. In regression analyses controlling for known risk factors such as female gender, cumulative trauma, severity of trauma (Criterion A), type of trauma (abusive event or not), ongoing adversity and low social support, negative appraisals were the best predictor of PTSD symptoms, explaining 22 % of the variance (Table 1). Results revealed that this relationship appeared specific to PTSD in adolescents, as negative appraisals did not predict internalizing symptoms ($\beta = 0.07$, $p = 0.23$).

Table 1 Hierarchical Multiple Regression Estimates for Predictors of Current PTSS

Independent variable	B	SE B	β	ΔR^2	adjusted R^2	Overall F
Model				.015**	.488	53.37***
Gender	0.48	.14	.14**			
Criterion A event	0.54	.16	.13**			
Abusive event	0.07	.20	.01			
Cumulative trauma	0.13	.04	.14**			
Negative appraisals	0.14	.01	.19**			
Ongoing adversity	0.05	.02	.12**			
Social support	-	.01	-.04			

Note. The dependent variable is PTSD Symptom Severity (square-root transformed). Gender is coded 0 males, 1 females. The variables age and time since trauma was excluded as had no significant effects in preliminary models. * $p < .05$; ** $p < .01$; *** $p < .001$

Association of daily stressors to PTSD symptoms, EBP and functional impairment

There were 474 adolescents (62.9%) reported having daily stressors. Most frequently reported daily stressors were financial problems, health problems, and problems in peer relations. As expected, daily stressors were positively correlated with total trauma exposure, PTSS and mpairment, and negatively correlated with social support. Daily stressors significantly predicted PTSD symptoms ($\beta = 0.33$, $p < 0.001$) and functional impairment ($\beta = 0.36$, $p < 0.001$). Figure 2 depicts the extent to which daily stressors indirectly transmitted the effects of trauma on PTSS and impaired daily functioning (models i, ii, and iii). For PTSS, the indirect effect accounted for 26.1% of the total effect of trauma (unstandardized coefficient $ab = 0.739$, 95% CI [0.459, 1.122]) and for functional impairment it was 70.0% ($ab = 0.072$, CI [0.049, 0.121]). Although mediation cannot be established in these cross-sectional data, further analysis showed that the pathways between daily stressors and mental health problems are likely bidirectional but stronger for the causal pathway from daily stressors to mental health outcomes than vice versa.

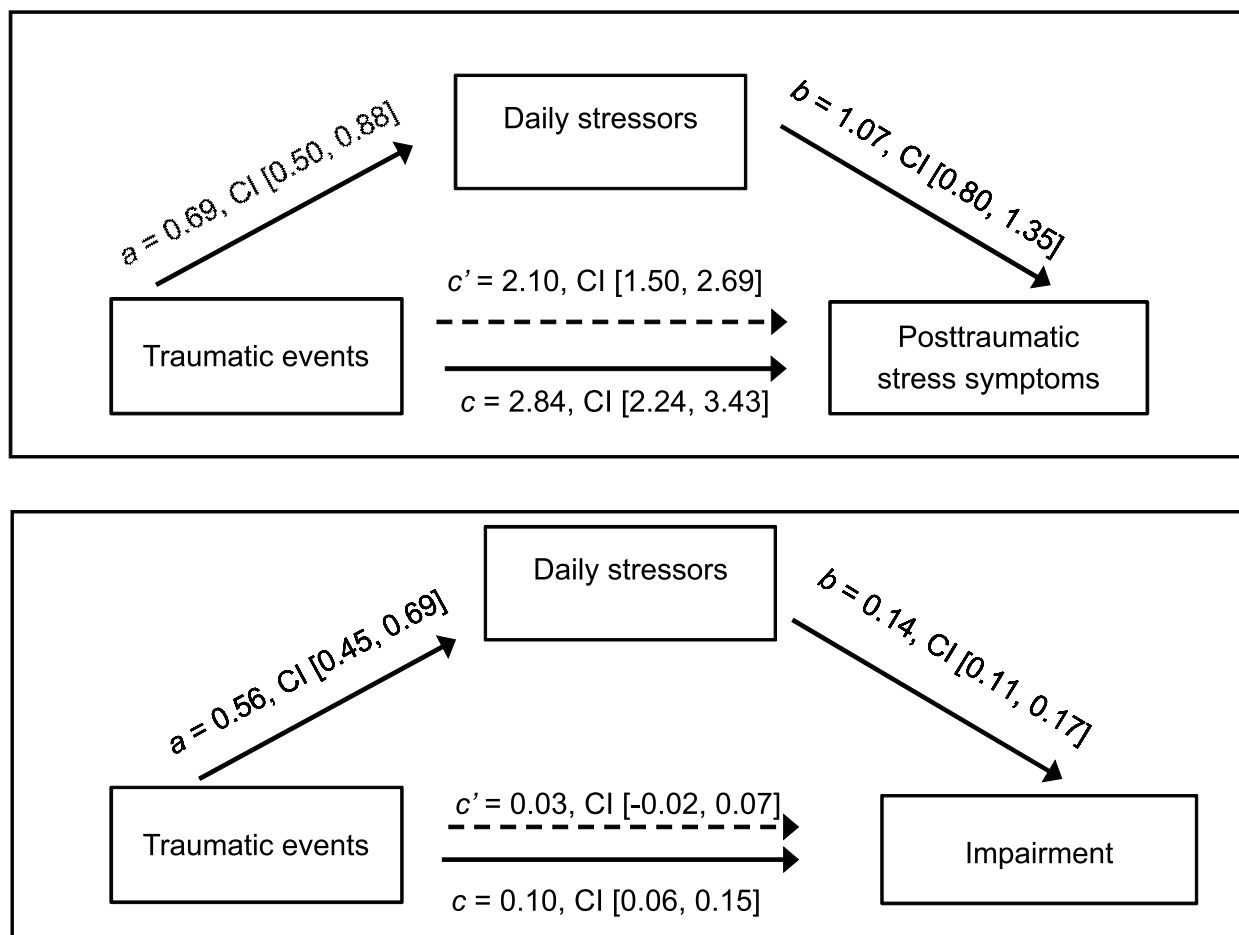


Figure 2. Unstandardized path coefficients for models in which daily stressors were hypothesized to transmit indirect effects of cumulative trauma on mental health outcomes. Models i, ii, and iii controlled for age, gender, and age by gender interaction; Model iii also controlled for EBP score. Coefficient a represents the effect of trauma on daily stressors, b is the effect of daily stressors on a given mental health outcome, c represents the total effect of prior trauma, c' is the direct effect of trauma after controlling for daily stressors. Indirect effect (via daily stressors) = $c - c' = ab$.

influenced path a, from trauma to stressors and also attenuated the relationship between stressors and outcome, path b (IMM = -0.005, CI [-0.010, -0.001]).

Association of basal cortisol level, diurnal slope and cortisol awakening response to trauma exposure and PTSD symptoms Results indicated higher cortisol in girls ($p = .042$) and older adolescents ($p = .002$). Controlling for age, gender and BMI results indicated no significant main effects of trauma or PTSD symptoms on overall cortisol levels or diurnal slope. Significant moderation by gender is observed in all three cases. Thus, girls with greater trauma exposure

or more severe PTSS showed elevated cortisol levels relative to similarly exposed or symptomatic boys (table 2). Co-occurrence of internalizing symptoms and PTSS was also associated with higher cortisol. Girls were more likely than boys to display elevated cortisol in relation to re-experiencing and hyperarousal symptoms. In contrast to significant findings for cortisol level and diurnal slope, the CAR showed no association with either trauma or PTSS, irrespective of gender.

Co-occurrence of EBP with PTSD

Symptom levels of SDQ total score (EBP total) and all four subscale scores of emotional, conduct, hyperactivity and peer problems were significantly high in PTSD positive adolescents (all $p < 0.001$). Post-hoc comparisons revealed higher scores among the PTSD+ group compared to the other two groups, whereas scores of the PTSD- and control group did not differ significantly. This was supported by the finding of caseness of EBP (abnormal to borderline symptoms) with PTSD (Table 3). The percentage of

individuals at risk were two-and-a-half times higher in the PTSD+ group compared to PTSD-; the PTSD- group, however, did not differ significantly from the controls. Bonferroni-corrected post-hoc comparisons confirmed a higher proportion of individuals at risk in the PTSD+ group. Multiple regression analysis revealed that EBP significantly associate with PTSD symptoms ($\beta = -0.24$, $p < 0.001$) after controlling for age, gender, and cumulative trauma exposure. PTSS and EBP were also independently increase impairment in adolescents (PTSS $\beta = -0.27$, $p < 0.001$, EBP $\beta = -0.20$, $p < 0.001$).

Table 2 Multilevel regression estimates for effects of trauma exposure and PTSS on salivary cortisol levels, as moderated by gender

	β	SE	Z	p	[95% CI]
Intercept	0.750	.088	8.48	< .001	[0.577, 0.923]
Control variables					
Time of day (hr)	-0.152	.006	24.02	< .001	[-0.164, -0.139]
Age (yr)	0.092	.090	1.03	.305	[-0.084, 0.269]
Gender	0.236	.115	2.04	.041	[0.009, 0.462]
Age x Gender	0.159	.120	1.33	.184	[-0.076, 0.394]
(a) Cumulative trauma					
Trauma (main effect)	0.003	.032	0.10	.918	[-0.060, 0.067]
Gender (main effect)	0.238	.120	2.02	.044	[0.007, 0.469]
Trauma x Gender	0.161	.070	2.31	.021	[0.024, 0.298]
(b) PTSS					
PTSS (main effect)	-0.002	.013	0.15	.879	[-0.026, 0.023]
Gender (main effect)	0.238	.118	2.02	.043	[0.007, 0.469]
PTSS x Gender	0.038	.014	2.72	.007	[0.010, 0.065]

Note. Estimates for fixed effects are based on 685 logcortisol measurements nested within 84 participants. Because results of preliminary multilevel models separately estimating effects on cortisol of BMI, menarcheal status, perceived SES, and current daily stressors did not approach significance (p values > 0.10), these variables were excluded from final models. Models a and were estimated separately, controlling for time, age, gender (0 male, 1 female), and Age x Gender

Table 3. SDQ risk categories in relation to trauma exposure and current PTSD

SDQ scale	Total		PTSD+		PTSD-		Notrauma		Test statistics
	N	%	n	%	n	%	n	%	
Total									
Normal	631	86.6	73	70.2	295	88.3	263	90.4	$X^2 = 28.48, p < 0.001$
At risk	98	13.4	31	29.8	39	11.7	28	9.6	[#] $a > b^{***} > c$
Emotional									
Normal	636	87.2	69	66.3	300	89.8	267	91.7	$X^2 = 48.12, p < 0.001$
At risk	93	12.8	35	33.7	34	10.2	24	8.3	[#] $a > b^{***} > c$
Peer									
Normal	591	79.8	81	77.9	270	80.8	240	82.5	$X^2 = 1.07, p = 0.585$
At risk	138	20.2	23	22.1	64	19.2	51	17.5	[#] $a > b > c$
Conduct									
Normal	642	88.0	81	77.8	295	88.7	266	91.4	$X^2 = 13.37, p = 0.001$
At risk	87	12.0	23	22.2	39	11.7	25	8.6	[#] $a > b^{***} > c$
Hyperactivity									
Normal	665	91.2	84	80.7	311	93.1	270	93.1	$X^2 = 16.57, p < 0.001$
At risk	64	8.8	20	19.3	23	6.9	21	6.9	[#] $a > b^{***} > c$

[#]Post-hoc comparisons between: PTSD+, PTSD-, No trauma; *** $p < .001$.

Discussion

This study had several aims. One is to identify traumatic event exposures and to examine the association of trauma exposure and negative appraisal to post-trauma mental health problems. Second is to identify daily stressors, and their implications for mental health and daily functioning. Third to examine the association of basal cortisol level, diurnal slope and cortisol awakening response to trauma exposure and post-trauma mental health problems. Finally, to investigate the co-occurrence of general mental health problems with PTSD symptoms in adolescents.

Negative appraisal and PTSD symptoms

Negative cognitive appraisals of trauma and/or its sequelae have been associated with PTSD in previous research. Surprisingly little is known as to what causes a trauma-exposed adolescent to develop negative cognitions concerning self and the world. Results revealed that the severity of the threatening event (criterion A event), events involving interpersonal trauma, lifetime trauma exposures, and current life difficulties were important risk factors for persistent negative trauma appraisals. This is in line with

evidence that negative cognitive appraisals play a role in chronic PTSD in adolescents. This new information adds to and extends the cognitive model of PTSD as formulated by Ehlers & Clark (2000).

Supporting the cognitive model of PTSD (33) and in line with the few studies that had previously investigated this model in younger populations, (34) our study demonstrated that negative cognitive appraisal of trauma was the best predictor of PTSS, even after controlling for other known risk and resilience factors. Further analysis showed that negative appraisal had no independent association with internalizing symptoms, thus the association of negative appraisals to PTSS was specific. Such findings about predictors of negative appraisal and its specific association to PTSS may help clinicians to design and apply more effective interventions in adolescents, as previous studies have shown that cognitive behavioral therapy leads to clinical improvement primarily through changing maladaptive appraisal (35).

Daily stressors and Post-trauma Mental Health Problems

Trauma exposure and daily stressors were significant predictors of current mental health problems and functional impairment. In this association, daily stressors played a mediating role in the pathway, transmitting part of the negative impact of traumatic stress on outcomes PTSS and functional impairment. Based on previous studies, we expected that the indirect effect would vary according to the specific mental health outcome (36). For PTSS, trauma exposure had a larger direct effect and a smaller indirect effect (26%). In impairment, trauma exposure had a smaller direct effect, and a larger indirect effect on outcomes (For, Functional impairment 70%). Therefore, traumatic stress was mediated to a lesser extent by daily stressors in trauma-specific mental health problems, but in trauma-nonspecific psychopathologies, much of the traumatic stress was mediated via daily stressors. However, this pathway can be bidirectional. Further, analysis showed that mental health problems did mediate the trauma – daily stress pathway, but the trauma to symptoms pathway, via daily stressors, explained more variance. Previous cross-sectional studies investigating daily stressors as a mediator of the trauma - psychopathology relationship did not consider whether current psychopathology might have increased daily stressors. This study is novel in this regard, having explored the likely direction of effects in a prospective subsample.

In line with previous studies, this study also provided evidence that social support could directly reduce mental health outcomes and also buffer the impact of trauma and subsequent life stressors on the development of PTSS. Further, in functional impairment, social support buffer the effect of trauma and daily stressors by weakening the indirect effect of trauma through daily stressors.

Biological stress system linked to trauma exposure and post-trauma mental health problems

The follow-up sub-sample investigate the HPA dysregulation in PTSD in adolescents. Contrary to our expectation, we observed no effect of either trauma exposure or PTSS on overall cortisol level, diurnal slope or cortisol awakening response. However,

further analyses pointed to a robust gender differences in the association of cortisol secretion with trauma and PTSS, with girls displaying higher cortisol levels than similarly affected boys. Our findings are in line with the recent meta-analysis which concluded that trauma-exposed females are more likely to show HPA hyperactivity. Although it is known that women are more vulnerable than men to PTSD, possible sex differences in the psychobiology of the disorder have received little research attention. This study thus attempted to explain the observed gender difference in HPA axis function in PTSD extended the available knowledge.

PTSD symptoms and co-occurring Emotional and Behavioural Problems

Comorbid psychiatric disorders are commonly noted among individuals with PTSD. In the present study, a considerable number (7%) of trauma-exposed adolescents met criteria for both PTSD and EBP. By comparing the EBP symptom level and EBP caseness between the three groups of PTSD-positive, PTSD-negative, and non-trauma exposed groups, we confirmed that PTSD+ participants experienced more EBP than the others. Our findings are in line with those of previous studies that confirmed that EBP are more closely associated with PTSD than with trauma exposure in adolescents (37). Further, the regression analysis demonstrated that PTSS increase EBP in adolescents, and PTSS and EBP were independently associated with greater impairment. The present study examining EBP caseness provided with more clinically important evidence. EBP (at risk range of SDQ) in PTSD positive adolescents might indicate the coexistence of other psychiatric morbidities, which need to be further assessed and treated (38).

Conclusion

Negative cognitive appraisal is the strongest factor that is associated with PTSD symptoms. This highlights the importance of addressing maladaptive cognitions in post trauma mental health problems. Daily stressors appeared to be an important transmitter of the impact of trauma on adolescents' mental health problems. That social support moderated direct and indirect pathways from trauma to daily impairment is a novel finding that warrants further investigation. Although longitudinal studies are needed in order to develop evidence-based interventions, current results

reinforce the argument that research and interventions for trauma victims should focus on ongoing stressors as well as on the total burden of trauma, also taking protective factors into account.

The observed robust gender differences in associations of trauma, PTSD symptoms with patterns of daily cortisol secretion support the notion of gender-specific increases in the sensitivity of the HPA system to trauma during mid-adolescence. This finding point that prospective studies are needed to determine the causal relationship and the knowledge will help to design effective prevention and treatment strategies in future.

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High-throughput screening for drug discovery: Exploring silent microbial secondary metabolites as an untapped molecular resource

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Abstract

High-throughput screening is a method which utilizes robots, detectors and software to accelerate the discovery of suitable drug targets and to facilitate early elimination of unsuitable compounds. High-throughput screening plays an important role in early stage of drug development providing qualitative and quantitative characterization of compound libraries and analytical support for preclinical and clinical studies.

This study is focused on high-throughput screening of microbial secondary metabolites produced as stimulatory responses when microbes are exposed to different conditions. The main goals were (i) investigating of plausible stimulation mechanisms, (ii) detection, characterization and evaluation of biological activity of novel secondary metabolites and (iii) investigating of plausible biosynthetic pathways of detected novel compounds.

High-throughput microscale cultivations were employed to probe the secondary metabolite potential on a set of microorganisms (×48) exposed to different conditions (×48). Obtained crude extracts were subjected to high-throughput screening using UPLC-DAD and HPLC-DAD-MS. Online SciFinder database and in-house Capon Group database assisted dereplication was performed for the detection of novel secondary metabolites. Further purifications were carried out focusing on novel, stimulated or low-yield/minor metabolites using semi preparative and preparative HPLC. Chemical characterizations of isolated metabolites were supported by detailed spectroscopic analysis, chemical derivatization methods and computational methods.

The following sections describe (i) LPS stimulated

secondary metabolites and the plausible mechanism of stimulation, (ii) prospection of hydroxamate coprogen siderophores in iron chelation therapy for the treatment of iron overload and (iii) chemical characterization, investigation of biosynthesis pathway and evaluation of biological activity of new nitro *depsi*-tetrapeptide diketopiperazine waspergillamide A.

Introduction

Microbes (fungi and bacteria) are a valuable source of secondary metabolites, featuring novel and diverse chemical structures, rich in carbocycle, heterocycle, stereochemical and functional group complexity.(1) Most importantly, many of these metabolites exhibit potent and highly selective biological properties that have inspired and fueled the pharmaceutical revolution that delivered the quality of life provided by modern healthcare. Microbe-inspired therapeutics found application for the treatment of a range of health issues, including microbial infections, cancer, inflammation, hypercholesterolemia and tissue rejection in organ transplantation. (2, 3)

Notwithstanding these successes, late last century key sectors in the pharmaceutical industry concluded (incorrectly) that the discovery of new microbial chemistry had peaked, and that the increased cost and delays represented an unacceptable risk and burden. As a consequence, many major pharmaceutical companies abandoned microbe inspired drug discovery, turning instead to synthetic chemical libraries. Unfortunately, the last two decades has provided ample evidence that the decision to abandon microbes was premature, with the synthetic alternative failing to live up to expectations. Society at large is now faced with a drug discovery pipeline

(drug-lead candidates currently under evaluation for commercial release) that is at its lowest level in many decades. In the field of antibiotics, we are faced with the very real prospect, for the first time in history, that the next generation will enjoy a lower level of effective therapeutic intervention than their parents. (4,5)

Fortunately, there is a potential solution, with microbial drug discovery poised for a renaissance. An explosion in genomic sciences has revealed that microbes (fungi and bacteria) have far greater capacity to produce natural products that was previously imagined, with multiple examples of “silent” secondary metabolism gene clusters. (5-9) Such “silent” chemistry, if accessed, has the potential to recharge the drug discovery pipeline. Furthermore, our capacity to access this “silent” potential has been radically advanced by improvements in microbiology (new isolation and cultivation technologies), chemistry (new analysis, spectroscopic and synthetic technologies) and biology (new disease targets and assays).

The flow of genetic information from DNA to RNA to proteins is controlled by a collection of interwoven regulatory networks that are responsible for affording cells control over transcriptional and translational processes. These essential mechanisms restrict the expression of the total genetic potential of an organism and as a result, many secondary metabolites pathways remain “silent” under normal culture conditions.¹⁰ While detailed understanding of these regulatory mechanisms and the factors that control them would accelerate the discovery and exploitation of silent/cryptic secondary metabolites, given the complexity of the processes this is not an easy task. That said, a metabolism-remodeling approach in which exogenous chemicals used at low concentrations can alter the secondary metabolites produced by microbes could lead to the discovery and characterization of a multitude of novel compounds that can act as leads in the drug discovery process.

This study is focused on stimulating the expression of microbial silent/cryptic secondary metabolites by culturing microbes in different culture media (agar, broth and rice grains), different culture conditions (liquid shake and static) and in the presence of chemical cues/stimuli (LPS, DKP, epigenetic modifiers etc.). For this investigation, a sub library of

Fungi (×33) and *Streptomyces* sp. (×15) was recovered from our in house Australian Collection of Microbes (ACM) and Capon Microbial Biodiversity (CMB) microbial collections. Secondary metabolite potential of all the microbes (×48) were screened in the presence of chemical cues/stimuli (×10). To further diversify the secondary metabolite potential, three wasp-derived fungal strains (CMB-W001, CMB-W031 and CMB-W045) were selected. These fungal strains were cultured under 38 different culture media conditions. Explored culture media and conditions include agar media (×12), liquid broth static condition (×12), liquid broth shake condition (×12) and rice grains (×2). Chemical profiling (UPLC-DAD and HPLC-DAD-MS) on the obtained microbial crude extracts led us to identify promising stimulatory events. Identified hits were subjected to scale up cultivations followed by isolation and purification using a range of methods (trituration, SPE columns, preparative HPLC and semi-preparative HPLC). Structure elucidation of isolated metabolites was supported by NMR (1D and 2D), HRESIMS, LC-MS, UV, optical rotation and circular dichroism (CD). Chemical derivatization methods (acid hydrolysis and C₃ Marfey's method) supported the assigning of absolute configurations. The biological activities of isolated pure compounds were screened under a range of biological assays [antimicrobial, cytotoxic, *Bacillus Calmette-Guérin* (BCG), nematicidal, iron chelation and antioxidant].

Materials and Methods

Solvents used for extraction and solvent-solvent partition of crude extracts were of analytical grade. For HPLC purifications, HPLC grade solvents were used after filtering and degassing through 0.45 µm polytetrafluoroethylene (PTFE) syringe filters. Deionised water was filtered through Elga PURELAB Ultra filtration system and degassed before using in HPLC instruments. Pre-prepared culture media were obtained from BBL/Difco. A pure sample of commercial lipopolysaccharide (LPS) isolated from *Escherichia coli* (0111:B4). Other chemicals were purchased from Sigma-Aldrich, Merck and Fluka. The nitric oxide detection kit (ENZ-51013) including the NO scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (c-PTIO) and matching 10X wash buffer was purchased from Enzo Life Sciences.

Equipment

Chiroptical measurements ($[\alpha]_D$) were obtained on a JASCO P-1010 polarimeter in a 100 × 2 mm cell. NMR experiments were performed on Bruker Avance 600 MHz spectrometer, in the solvents indicated and referenced to residual ^1H signals in the deuterated solvents. Electrospray ionization mass spectra (ESIMS) were acquired using an Agilent 1100 Series separations module equipped with an Agilent 1100 Series LC/MSD mass detector in both positive and negative modes. High-resolution (HR) ESIMS measurements were obtained on a Bruker micrOTOF mass spectrometer by direct infusion in MeCN at 3 L/min using sodium formate clusters as an internal calibrant. Liquid chromatography-diode array-mass spectrometry (LC-DAD-MS) data were acquired on an Agilent 1100 series separation module equipped with an Agilent 1100 series LC/MSD mass detector and diode array multiple wavelength detector. Ultra high-performance liquid chromatographs (UPLC) were obtained on an Agilent 1290 infinity UHPLC system composed of 1290 infinity quaternary pump, thermostat, autosampler and diode array detector. Preparative HPLC was performed using Agilent 1100 with corresponding detectors, fraction collectors and software inclusively. Semi-preparative HPLC was performed using Agilent 1100 series LC instruments with corresponding detectors, fraction collectors and software inclusively.

Microorganisms were cultured under sterile conditions providing by a Laftech class II biological safety cabinet and incubated in MMM Friocell incubator or Innova 42 incubator shaker with temperature set at 26.5 °C. Pure strains were cryo-preserved in 15% glycerol at - 80°C in ilShin deep freezer. Twenty-four well micro-bioreactor, cover clamp and sandwich covers were obtained from Applikon Biotechnology.

Confocal microscopy samples, in 12 mm coverglass bottom culture dishes purchased from (ProSciTech), were excited with a 543 nm He Ne laser and measured emissions filtered through a 560 nm long-pass filter.

Taxonomy of Fungal Strain CMB-W045 and CMB-W031

Genomic DNA from this isolate was extracted from the mycelia using the DNeasy Plant Mini Kit

(QIAGEN) as per the manufacturers protocol. The rRNA genes were amplified by PCR using the universal primers ITS 1 and ITS 4 purchased from Sigma-Aldrich. The PCR products were purified with PCR purification kit (QIAGEN) and sequenced. ITS DNA sequence (~800 bp) was subjected to GenBank BLAST.

Phylogenetic Tree

PhyML Maximum Likelihood analysis of CMB-W045 and CMB-W031 ITS DNA sequences were performed using optimal nucleotide substitution model JC69 (jModeltest2). UGENE was used to construct and view the phylogenetic tree.

Analytical scale chemical profiling of crude extracts HPLC-DAD-MS analysis of crude extracts Aliquots (10 µL) of crude extracts were analyzed on Agilent 1100 series LC/MSD instrument with Zorbax C₈ column (150 × 4.6 mm, 5 µm) with UV-vis detection at 210, 254, 360, 400 and 550 nm). LC-MS ChemStation software was used in analyzing HPLC-DAD-MS profiling data.

Scale-up fermentation, isolation and characterization of LPS stimulated metabolites 1 – 5.

Six Fernbach flasks (2 L) containing SDB (495 mL) were inoculated with the seed culture (5 mL) of *Penicillium sp.* (ACM-4616). Five flasks were treated with LPS (0.6 ng/mL) were incubated at 26.5 °C for 7 days. The resulting cultures were extracted with EtOAc (2.5 L) and the combined organic phases concentrated in vacuo to yield a total extract (165.3 mg). The total extract was sequentially triturated (8 mL aliquots) and concentrated in vacuo to yield hexane (4.0 mg), CH₂Cl₂ (159.4 mg) and MeOH (1.9 mg) soluble fractions. The CH₂Cl₂ soluble fraction was subjected to preparative reversed-phase chromatography on a Phenomenex Luna C18 10 mm (250 × 21 mm) column to yield cyclo-(L-Phe-L-Pro) (**1**) (3.3 mg, 1.99%), cyclo-(L-Trp-L-Pro) (**2**) (0.9 mg, 0.54%), pseurotin A (**3**) (2.0 mg, 0.72%), pseurotin A₁ (**4**) (1.2 mg, 0.72%) and pseurotin A₂ (**5**) (0.9 mg, 0.54%). The % yields were determined on a mass-to-mass basis against the weight of the total extract.

Scale-up Fermentation, Isolation and Characterization of 9–16

Forty 250 mL Erlenmeyer flasks containing sterile jasmine rice (20 g) inoculated with homogenized suspension (5 mL) of CMB-W045 were incubated at 26.5 °C for 15 d, after which the rice was extracted with acetone (3x3400 mL) and concentrated in vacuo at 40 °C to afford the crude extract (63.4 g). The crude extract was partitioned between EtOAc (2x1150 mL) and H₂O. The obtained EtOAc partition was triturated to yield hexane (2x300 mL) (3.84 g) and MeOH (300 mL) (1.87 g) soluble partitions.

The aqueous layer was subjected to C₁₈-SPE fractionation and 50% MeOH soluble fraction (1.0 g) was obtained. From the 50% MeOH soluble fraction (260 mg) was subjected to preparative HPLC (Phenomenex Luna – C₁₈ column) to yield 19 fractions. Fraction A and B were subjected to semi preparative separation (Zorbax SB CN column 9.4 mm 2.5 cm, 5µm) to dimerumic acid (**14**) (2.4 mg, 16.9 %), talarazine A (**9**) (1.5 mg, 10.5 %), elutherazine B (**15**) (0.8 mg, 5.6 %), desferricoprogen (**16**) (3.8 mg, 13.2%), talarazine D (**12**) and talarazine E (**13**) mixture (2.4 mg, 8.3 %), talarazine C (**11**) (1.9 mg, 6.6 %) and talarazine B (**10**) (0.9 mg, 3.1 %). The % yields determined on a mass-to-mass basis against the weight of the aqueous partition.

C₃ Marfey's analysis

Samples of diketopiperazines 1–2, 9–16 (50 µg) in 6 M HCl (100 µL) were heated to 110° C in sealed vials for 24 h, after which hydrolysates were evaporated to dryness at 40 °C under a stream of dry N₂. The resulting hydrolysates were treated with 1 M NaHCO₃ (20 µL) and L-FDAA (1-fluoro-2-4-dinitrophenyl-5-L-alanine amide, 1% solution in acetone, 50 µL) at 40 °C for 1 h. Samples were neutralized with 1 M HCl (20 µL), diluted with MeCN (810 µL) and analyzed by HPLC-ESIMS (Agilent Zorbax SB-C3 column, 5 µm, 150 × 4.56 mm). Authentic amino acids L-Phe, L-Trp and L-Pro were derivatized with L-FDAA and analyzed with the HPLC-ESIMS. Authentic amino acid D-Orn was derivatized with D-FDAA and L-FDAA analyzed with the HPLC-ESIMS.

NO assay

The production of nitric oxide (NO) was detected using a NO detection kit. Briefly, four fungal strains

were chosen for the detection of NO production, including the LPS responsive *Penicillium* sp. (ACM-4616) and *Penicillium* sp. (CMB-TF411), and the LPS non-responsive *A. brasiliensis* (ACM-4711) and *A. oryzae* (ACM-4669). Inocula were prepared and the strains were cultivated in the micro-bioreactor for 7 days at 190 rpm, 27 °C in ISP-2 broth. To detect the production of NO, fungal cells were washed twice with 10× wash buffer composed of normal saline neutral (pH 7.3) phosphate buffered saline, after which they were centrifuged at 14,000 rpm for 1 min and the cells transferred to cover glass (12 mm) bottom plates. The NO detection reagent was diluted by adding the reagent (2.5 µL) to ISP-2 broth (1 mL), after which 100 µL was added to the fungal cells. Following the addition of the NO detection reagent the plates were incubated for 2 hours at 27 °C, after which LPS (15 µL, 0.6 ng/mL) was added and the plates were incubated at 27 °C for a further 30 min. In addition to treating cells with LPS, duplicate sets of two forms of negative control were prepared where cells were (i) not treated with LPS and (ii) were treated with LPS (15 µL, 0.6 ng/mL) but were subsequently treated with the NO scavenger c-PTIO (40 µM, 100 µL) and incubated for a further 15 min. The fluorescent products from the NO detection kit were measured by confocal microscopy and the images analyzed and processed using ImageJ-145 software and NOS-Elements AR version 3.2.

Antimicrobial assay

Antimicrobial activities were measured against Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923), Gram-negative bacteria *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and a fungus *Candida albicans* (ATCC 90028) by the broth micro-dilution method. The test was performed (in duplicate) in 96-well microtiter plates. Test compounds were prepared and serially (ten-fold) diluted in 10% DMSO. An aliquot (20 µL) of each dilution was transferred to a 96-well microtiter plate, followed by freshly prepared microbial broth (180 µL, 104–105 cfu/mL cell density) to give a final test compound concentration ranging from 32 to 0.125 µg/mL. The plates were incubated at 37 °C for 24 h for bacteria and at 26.5 °C for 48 h for yeast. The optical density of each well was measured at 600 nm using a microtitre plate spectrophotometer (POLARstar Omega plate, BMG LABTECH, Offenburg,

Germany). Broth medium with and without microbial inoculation were used as negative controls, with tetracycline and ketoconazole used as positive controls for antibacterial and antifungal assays, respectively. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of a test compound that inhibits 90% of microorganism growth.

Cytotoxicity assay

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method was used for in vitro evaluation of the cytotoxic activities of pure compounds against human lung cancer (NCI-H460), human colon adenocarcinoma (SW-620) and human cervical carcinoma (KB-3-1) cell lines. Cells in 180 μ L RPMI media (1×10^5 – 4×10^5 cells/mL) supplemented with 10% heat-inactivated foetal bovine serum were seeded in 96-well microtiter plates, at 2,000 cells/well and incubated for 18 h at 37 °C under 5% CO₂ for cell attachment. Test compounds were dissolved in 10% DMSO in PBS and aliquots (20 L) tested over a series of final concentrations ranging from 30 μ M to 10 nM in duplicate in the plates. Negative control wells contained 10% aqueous DMSO without any compound. After 68 h incubation at 37°C with 5% CO₂, an aliquot (20 L) of MTT in PBS (4 mg/mL) was added to each well (final concentration of 0.4 mg/mL), and the microtitre plates incubated for a further 4 h at 37°C with 5% CO₂. After this final incubation, the medium was aspirated and precipitated formazan crystals dissolved in DMSO (100 L/well). The absorbance of each well was measured at OD_{580 nm} at r.t. on a POLARstar Omega microtitre plate reader. IC₅₀ values were calculated using Prism 5.0 (GraphPad Software Inc., La Jolla, CA), as the concentration of analyte required for 50% inhibition of cancer cell growth (compared to negative controls). All experiments were performed in duplicate.

Bacille Calmette-Guérin (BCG) Assay

Mycobacterium bovis Bacillus Calmette-Guérin (BCG) (ATCC 35734, Pasteur) was grown at 37 °C to mid-log phase in Middlebrook 7H9 broth containing 10% OADC (Oleic Albumin Dextrose Catalase) enrichment, 0.2% glycerol, 0.05% Tween 80, 0.5% bovine serum albumin (BSA), 0.2% dextrose and 0.085% sodium chloride. Single cell suspension of

BCG was prepared by centrifuging the bacterial cultures for 10 min, and then diluted with culture medium to OD₆₀₀ values of 0.02. Aliquots (195 μ L) of the bacterial suspension were added to each well of flat-bottom 96-well microtiter plates, followed by supplying two-fold serially diluted pure compounds (prepared in 2.5% DMSO, 5 μ L per well) in duplicate with the final concentrations ranging from 30 μ M to 30 nM. The 96 well plates were incubated at 37 °C with 5% CO₂ for 7 d. After the incubation period, the optical density of each well was measured spectrophotometrically at 600 nm. The MIC was defined as the minimum concentration of compound that inhibits more than 90% of bacteria growth.

Calcein-Fe (CAFe) Assay

Testing compounds **9** – **16** were prepared and serially diluted in DMSO (0 to 1 mM), and an aliquot (5 mL) of each dilution was dispensed into 96-well, flat microtiter plates in duplicate. An aliquot (95 mL) of 2 mM calcein-Fe complex (CAFe) in HBS:DMSO (50:50, v/v) was then dispensed into each well to give a final analyte concentration ranging from 0 to 50 mM. HBS (Hepes Buffered Saline) was prepared by dissolving HEPES (20 mM) and NaCl (150 mM) in distilled H₂O, washed with Chelex(R)-100 (0.01 g/mL) and finally adjusted pH to 7.4. CAFe was prepared by dissolving FeSO₄ in aqueous calcein in order to reach a final concentration of 10 mM in both Fe and calcein. Assay plates were incubated at r.t. in the dark overnight, after which the fluorescence of each well was measured at r.t. using a POLARstar Omega microtitre plate reader (top reading; $\lambda_{exc}/\lambda_{em}$ = 485/530 nm; gain 850). A calibration curve was generated using desferrioxamine (DFO) (0 to 2 mM) as the positive control. The apparent binding constant (Kapp) was calculated from the following equation.¹¹

$$K_{app} = \frac{[CA][Fe(chelator)_3]}{[CAFe][chelator]_3 K_{diss}(CAFe)}$$

$$K_{diss}(CAFe) = 1.0 \times 10^{-24}$$

DHR Assay

Stock solutions were prepared; Ascorbic acid 8 mM in water (aliquots of 150 mL), Dihydrorhodamine 100 mM in DMSO (aliquots of 12 mL). HBS buffer (Hepes Buffered Saline; hepes 20 mM; NaCl 150 mL; Chelex® 1g/100 mL; pH adjusted to 7.4); 50 mL. An initial solution containing hepes and NaCl is prepared to the indicated concentrations, then washed with

Chelex®-100 resin by gently shaking. The resin was filtered off and the filtrate had its pH adjusted. Desferrioxamine (DFO) stock in HBS (50 mM; 1 mL), Nitrilotriacetic acid (NTA) 70 mM in water (5 mL). Fe(NTA) (iron nitrilotriacetate): 2.78 mg of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ were dissolved with 1 mL of 70 mM NTA and allowed to react overnight at r.t.. The solution turned to a pale yellow. Final $[\text{Fe}] = 10 \text{ mM}$. In any given well, in duplicates: Fe(NTA) (5 mL, 5 mM) in water, DFO (5 mL) (eg, 0–20 mM in HBS; calibration curve), or chelators **9–16** (5 mL) (as a serial dilution, eg 0–20 or 0–40 mM in DMSO), “fluorogenic cocktail” (90 mL) composed of DHR (50 mM) and ascorbic acid (40 mM) in HBS:DMSO 1:1. Fluorescence kinetics (485/530 nm; 37°C; gain = 1100) at 1 min intervals during 40 min readings were taken. The slope (fluorescence units/min) in the range 15–40 min was calculated.⁽¹²⁾

Results

LPS stimulated secondary metabolites and the possible mechanism of stimulation

Lipopolysaccharides (LPS) isolated from *Escherichia coli* (0111:B4) shows stimulatory effects on the fungal strains *Penicillium* sp. (ACM-4616) and *Penicillium* sp. (CMB-TF411).¹³ Preparative scale cultivation of *Penicillium* sp. (ACM-4616) followed by isolation and characterization confirmed an enhancement in production of the PKS-NRPS hybrid metabolite pseurotin A (**3**), as well as a concurrent activation of pseurotin A₁ (**4**) and pseurotin A₂ (**5**), and the diketopiperazines *cyclo*-(L-Phe-L-Pro) (**1**) and *cyclo*-(L-Trp-L-Pro) (**2**). Secondary metabolites stimulated upon treating with LPS (0.6 ng/mL) on *Penicillium* sp. (ACM-4616) are shown in Figure 1.

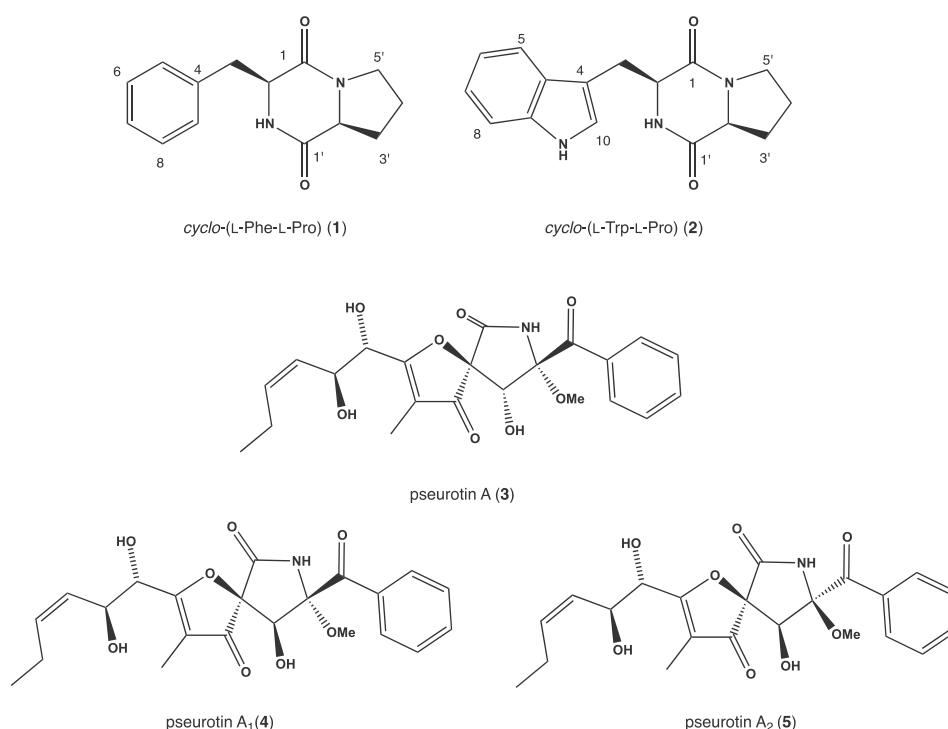


Figure 1. *Penicillium* sp. (ACM-4616) secondary metabolites (**1–5**) stimulated upon adding LPS (0.6 ng/mL)

UPLC-DAD analysis of LPS treated micro-bioreactor cultures of *Penicillium* sp. (CMB-TF411) revealed changes to the secondary metabolite profile. HPLC-DAD-ESIMS analyses and preparative scale cultures confirmed enhancements in (+)-deoxyluteoskyrin (**6**) ($[\alpha]_D + 85$) and (–)-rugluosin (**7**) ($[\alpha]_D - 22$) respectively, and activation of (–)-skyrin (**8**) ($[\alpha]_D - 16$). To the best of our knowledge (+)-deoxyluteoskyrin (**6**) and (–)-skyrin (**8**) have not been previously described (Figure 2).

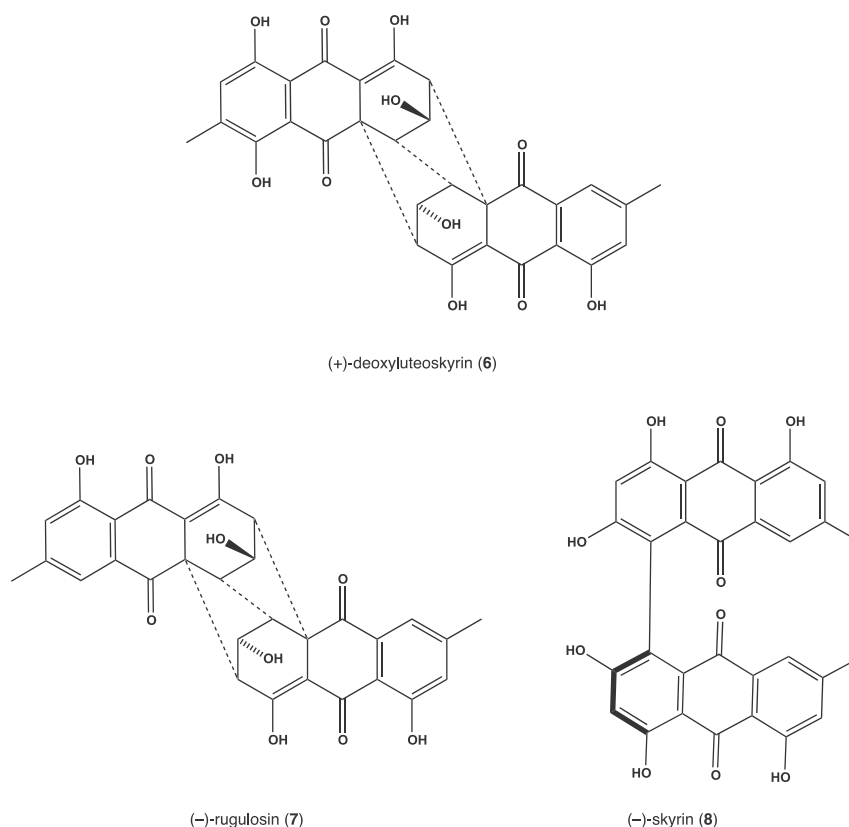


Figure 2. *Penicillium* sp. (CMB-TF411) secondary metabolites (6 – 8) stimulated upon adding LPS (0.6 ng/mL)

A 2011 study by Martin et al. describe the induction of “silent” pseurotin secondary metabolism in *A. fumigatus*, including an account of co-cultivation with a hyper-arid Atacama Desert soil *Streptomyces bullii*, (14) and an account on the impact of hypoxic cultivation (15). The latter report is noteworthy in so far as LPS is known to stimulate NO production in mammalian cells, which in turn inhibits cellular respiration leading to metabolic hypoxia. (16)

This suggested us a possible link between LPS inducing NO production and hypoxia and the LPS stimulated production of pseurotin A (3) – a secondary metabolite produced under oxygen limited/hypoxic conditions.

Light and fluorescent microscopy imaging of ACM-4616 and CMB-TF411 fungal cells treated NO sensitive fluorescent dye (i) with LPS, (ii) without LPS, and (iii) with LPS and NO quenching agent was performed. Significant levels of fluorescence was observed in the cells that were treated with LPS compare to that of the control. (13)

Both the fungal strains ACM-4616 and CMB-TF411 showed significant levels of fluorescence related to NO production compared to two fungal strains ACM-4669 and ACM-4711 that did not show any stimulatory response to LPS. These observations suggest a correlation between LPS stimulation of NO and triggering silent/cryptic secondary metabolites.

Prospection of hydroxamate coprogen siderophores in iron chelation therapy for the treatment of iron overload. Chemical analysis of an Australian mud dauber wasp-associated fungus, *Talaromyces* sp. (CMB-W045), yielded five new coprogen siderophores, talarazines A–E (9–13), together with dimerumic acid (14), and elutherazine B (15) and desferricoprogen (16) (Figure 3). Structures inclusive of absolute configurations were assigned on the basis of detailed spectroscopic analysis and application of the C3 Marfey's method. We report on the noncytotoxic Fe(III) chelation properties of (9–16).

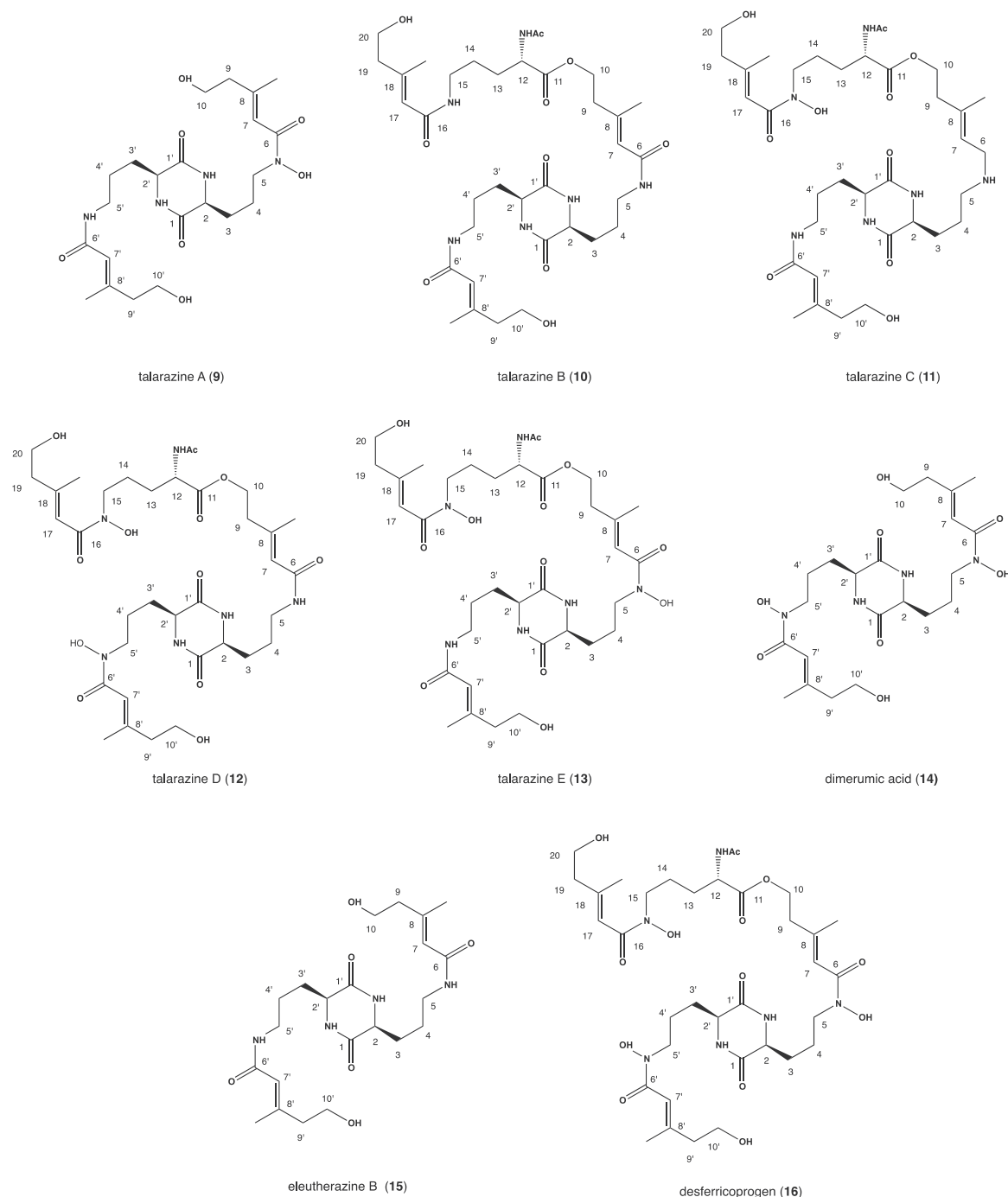


Figure 3. Structures of talarazines A – E (9 – 13) the new coprogen type siderophores and DKPs isolated from the *Talaromyces* sp. (CMB-W045).

The Fe (III) chelating capacity of 9–13 were investigated in the calcein-iron (CAFe) assay. Calcein undergoes fluorescence quenching upon binding of Fe (III). Upon interrupting the binding of Fe (III) to calcein, fluorescence is detected. In the CAFe assay, a competing equilibrium between Fe (III) chelators and the complex calcein-Fe (III) (CAFe) is used to assess the relative affinity of putative chelators of CAFe complex and releasing fluorescent calcein which is then quantified fluorometrically.(17) In CAFe assays, dimeric acid (14), desferricoprogen (16) and talarazine D/E (12,13) exhibited strong affinity to Fe (III).

In treating Fe (III) overload condition with chelation therapy, chelators are sought that are (i) able to penetrate

iron-overloaded tissue, (ii) coordinate iron in order to form stable and redox inactive species (thus decreasing intracellular labile iron pools), and (iii) transfer it to the circulatory transferrin. The CaFe assay demonstrated that **12** - **14** and **16** show strong affinity for Fe (III). Therefore, it was of further interest to find their antioxidant potentials. With the use of ascorbate as the redox-prompting agent, the detection of radical formation was assessed using an oxidant-sensitive fluorescent probe dihydrorhodamine 123 (DHR). Upon addition of chelators that has high affinity to Fe (III), the ROS decreases inhibiting the oxidation of DHR to rhodamine, which results in a decrease in the fluorescence. The rate of decrease in the DHR oxidation is highest for chelators with a high affinity for Fe (III). When **12** - **14** and **16** were tested in the DHR oxidation assay, the rate of decrease in fluorescence/DHR oxidation was observed in the order **16** > **14** > **12/13**. As a consequence, the antioxidant potential is in the order **16** > **14** > **12/13**.¹⁸

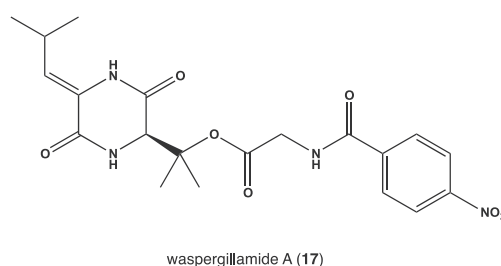


Figure 3. Structure of waspergillamide A (**17**)

Aromatic nitro groups while relatively rare, are found in diverse types of natural products, many of which exhibit biological properties.⁽¹⁹⁾ Two main pathways are possible for the formation of nitro compounds in nature include (i) nitration or (ii) oxygenation of amines. To differentiate between the two possible mechanisms for the biosynthesis of **17**, NOS inhibitor aminoguanidine (AG) was added to CMB-W031 cultures. Upon analyzing HPLC-DAD-MS data, it was observed that the production levels of **17** remained unaltered. Another experiment was conducted using fluorescent microscopy coupled with an NO selective and sensitive fluorescent dye. Obtained results confirmed that NO was not detectable in CMB-W031 cells under cultivation conditions capable of producing **17** with compared to the controls. These two independent studies confirm that the biosynthesis of **17** is not NOS or NO dependent.

Supportive of the above, on the basis of waspergillamide A (**17**) structure, we postulate it to be assembled by a modular non-ribosomal polyketide synthase (NRPS) pathway. Assembly starts with incorporation of *p*-amino benzoic acid (PABA) (the nitration taking place later in the biosynthesis) or *p*-nitro benzoic acid (PNBA) as the starter unit. The assembly then proceeds with sequential incorporation of amino acids glycine, 3-hydroxy-D-valine and leucine. Assembly terminates with an internal cyclization to form a 2,5-diketopiperazine ring (Figure 4).²¹

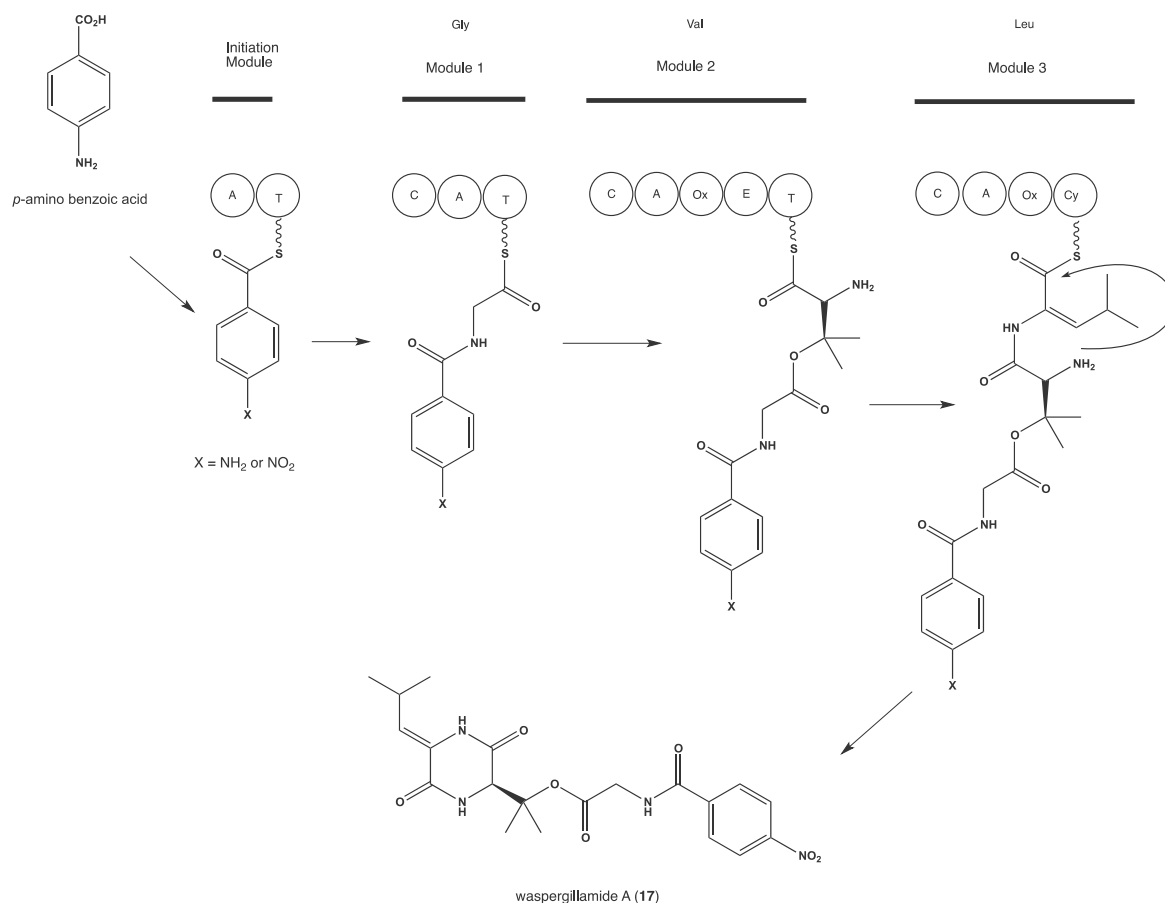


Figure 4. Model of waspergillamide A (17) biosynthesis

Conclusions

LPS stimulated secondary metabolites and the possible mechanism of stimulation.

The mechanism behind LPS stimulation was explored with the use of light and fluorescent microscopy imaging on fungal cells treated NO sensitive fluorescent dye. Fungal strains ACM-4616 and CMB-TF411 showed significant levels of fluorescence with compared to two fungal strains (ACM-4711 and ACM-4669) that did not show any stimulatory response to LPS. These observations suggest a correlation between LPS stimulation of NO and triggering silent/cryptic secondary metabolites. Triggering of secondary metabolite production may be correlated with activation of NO production suggestive of a chemical defense response.(13)

Prospection of hydroxamate coprogen siderophores in iron chelation therapy for the treatment of iron overload.

Five new and three known secondary metabolites from the wasp-derived fungal strain *Talaromyces* sp. (CMB-W045). These new metabolites include five new hydroxamate coprogen-type siderophores talarazine A - E (9 - 13). Known metabolites include two known coprogen type siderophores dimerumic acid (14), desferricoprogen (16) and a known diketopiperazine elutherazine B (15).

Compounds 9 – 13 were non-cytotoxic (IC₅₀ > 30 μM) (against SW-620, NCI-H460, KB-3-1 cancer cell lines) and non-antimicrobial (IC₅₀ > 30 μM) (against Gram negative bacteria *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), the Gram positive bacteria *Staphylococcus aureus* (ATCC 25923) and *Candida albicans* (ATCC 90028). The iron binding potentials and antioxidant potentials of 9 - 16 were investigated in CAFE and DHR

oxidation assays respectively. The results of CAFE and DHR oxidation assays confirmed the new metabolites talarazine D (12) and talarazine E (13) show moderate affinity to Fe(III) and moderate antioxidant potential with respect to desferrioxamine (16).

In both assays desferrioxamine was used as the standard chelator. Desferricoprofen (8), besides showing fluorescence recovery, revealed antioxidant capacity similar to desferrioxamine. In fact, both are tris-hydroxamate, which may complete all coordination sphere of iron. Comparing both assays, we conclude that desferricoprofen could be an interesting candidate for the chelation therapy of iron overload.

Chemical characterization, investigation of biosynthesis pathway and evaluation of biological activity of new nitro depsi-tetrapeptide diketopiperazine waspergillamide A.

Biosynthesis of 17 proceeds by a nonribosomal peptide synthase (NRPS) employing PABA as the starter unit. In this hypothesis PABA is sequentially conjugated with Gly, L-Val, and L-Leu residues, which are further elaborated by epimerization (L-Val to D-Val) and oxidation (PABA to *p*-nitrobenzoic acid (PNBA); L-Val to 3-OH-L-Val; L-Leu to $\Delta^{2,3}$ -Leu). The biosynthesis of 17 is concluded by 2,5-diketopiperazine cyclization-mediated termination. A failure to detect PNBA in CMB-W031 extracts supports the hypothesis that biosynthesis of 17 is initiated by PABA and not preformed PNBA. Disappointingly, supplementing CMB-W031 cultivations with PABA did not increase the yield of 17, nor was the biosynthetic pathway influenced by the addition of *p*-nitro, *o*-nitro, *m*-nitro, or *o,p*-dinitro benzoic acids.(21)

Waspergillamide A (17) is the first example of a natural diketopiperazine incorporating either a 3-OH-Val or a *p*-nitrobenzamide residue and, to the best of our knowledge, the first example of a natural depsi-tetrapeptide diketopiperazine. Importantly, natural nitro-substituted diketopiperazines are rare, with a noteworthy example being the phytotoxic thaxtomins produced by *Streptomyces* strains responsible for scab disease in potatoes.(22, 23) By contrast, waspergillamide A (17) was inactive in growth inhibition assays against a panel of Gram-negative and Gram-positive bacteria and human colon (SW620),

lung (NCI-H460), and cervical (KB-3-1) carcinoma cells.(21)

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Determinants and Characteristics of Candida colonization among patients admitted to ICUs of Teaching Hospital Karapitiya.

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Abstract

Candidaemia is an infection with a high mortality rate and the main reasons are delay in initiation of antifungal therapy or treatment with an ineffective antifungal. Ability to predict this infection gives a significant advantage for the clinician by pre-emptive treatment and reduce mortality. Aim of the study was to determine the level of colonization and correlated factors in a population at risk and to predict the risk of candidaemia,

This study was conducted on neonatal, paediatric and adult ICUs of Teaching Hospital Karapitiya. Screening specimens of oral, rectal, CV line swabs, urine and other available sterile fluids were collected from admission and every third day until discharge, death or development of Candidaemia in the patient. Candida Colonization index, Corrected Candida Colonization index and Candida score was calculated for each patient.

Colonization was found in 100 patients while 10 developed Candida blood stream infection. Presence of Sepsis, GI infection or surgical procedure, number of antibiotics given, CV line colonization, density of rectal, oral, urine colonization, and change of species of colonizing candida was significantly different among the infected and non-infected groups. Candida score had 100% sensitivity and the highest specificity of 94% was given by Corrected Candida Colonization index.

All the indexes and scores had excellent sensitivity and negative predictive values. However none of the scores had good specificity except for Corrected Candida colonization index.

Combination of Corrected Candida Colonization index and Candida Score could reliably predict Candidaemia.

Introduction

Candida is a yeast found as a commensal on the human gut and the skin. Mortality rate of 0.4 per 100,000 population was reported following Candida blood stream infection, which is similar to the rate of severe sepsis (1).

Candida is the commonest cause of fungal infection throughout the world and ranks at the fourth place among hospital acquired blood stream infections in studies done in USA (2).

The advancement of Medicine has increased the survival capabilities of patients and has resulted in an ever increasing cohort of patients on invasive lines and immunosuppressive therapy, and cancer patients who are fighting for life. HIV epidemic has also contributed to this rising trend of immunologically challenged patients. Use of broad spectrum antibiotics, transplantation of solid organ and haematopoietic stem cells has increased all over the world including in developing countries like Sri Lanka. Therefore the fungal infections have been growing throughout the world (1).

Risk factors for Candida blood stream infection has been studied extensively. Prolonged ICU or hospital stay is the commonest health care associated risk factor for Candida blood stream infection (3). Studies done in ICUs have revealed that there are two main risk factors for Candidaemia which are colonization with Candida and alteration of natural host barriers (4).

Pittet et al in 1994 formulated the Candida Colonization Index (5). It is calculated by dividing the number of sites of colonization by the total number of sites cultured. This formulation has been used successfully in prediction of Candidaemia in critically ill surgical patients. Additionally it was tested with variable results in medical patients. A meta- analysis on other studies done in different hospitals was

published by the same authors in 2014 and it revealed the success of prediction of Candidaemia by Candida Colonization Index (6).

Candida score is formulated by addition of clinical status of the patient by Leon et al in 2006. The score gave 1 mark each for multi focal colonization, parenteral nutrition, and surgery at ICU admission and 2 marks for sepsis. A score of more than 2.5 accurately predicted patients who will benefit from antifungal treatment (7).

Studies done by Eggimann in 2014 show that Candida score was more accurate than Candida colonization index in predicting Candidaemia (8).

Similar studies on Candida colonization have not been done in Sri Lanka to validate the Candida colonization index or Candida score for Sri Lankan population, which will help to predict the risk of Candidaemia in each patient. Aim of the study was to determine the level of colonization and correlated factors in a population at risk and to predict the risk of candidaemia

Materials and Methods

Specimens collected included swab from Oropharynx, Swab from rectum, swab from any Central lines, and dialysis catheters, and urine specimen. Additionally fluid from abdominal drains, IC drains were obtained when available.

Specimen collection was repeated every third day until the patient was discharged from the ICU or until the patient developed Candidaemia.

All the collected specimens were transported to the Microbiology laboratory of the Department of Microbiology within 2 hours and processed immediately.

The specimens were inoculated on to Candida Chromagar (Oxoid) as soon as possible.

Growth on each plate was noted at the end of 24 hours, 48 hours, 72 hours and 7 days. The colour of the colony was noted. Different types of colony morphology on the plates were noted. Growth of more than one type of species was also noted. Growth on each plate was quantified.

Each isolate was identified further by inoculating on

Corn meal agar with tween 80 to observe Chlamydospore formation and hyphal morphology and Yeast Nitrogen Base agar for carbon assimilation studies.

Antifungal susceptibility testing (AFST) was performed according to the Clinical and Laboratory Standards Institute (CLSI) method in the M44A2 document of August 2009.

Muller Hinton agar with added 2% glucose and 0.5µg/ml Methylene blue was used for the AFST.

Antifungal sensitivity testing was done using Fluconazole 25µg disks, Voriconazole 1µg disks and Amphotericin 10mg disks.

Interpretation of zone diameters was done for Fluconazole and voriconazole according to CLSI methods. Amphotericin B sensitivity was done as it was the commonly used systemic therapy even though CLSI zone diameters were not available. Interpretation was done according to manufacturer's recommendations. Posaconazole and Caspofungin sensitivity was not performed as the disks were not available in the country and no supplier had the licence to import disks to Sri Lanka.

Study design

The study was conducted on the patients admitted to Main ICU, Emergency treatment unit ICU, Oncology ICU, Neonatal ICU and Paediatric ICU of Teaching Hospital Karapitiya. It was conducted for a period of 4 months starting from 1st December 2015. It was a cross sectional analytical study design.

Sample size was determined using the equation for sample size calculation to estimate a mean value (Antonisamy 2010)

n = Sample number

σ = Assumed Standard deviation

Z = 5% significance level

d = Precision

Standard deviation was derived from the study done by Pittet D in 1994 which gave a value of 0.12 for the colonized patients and 0.1 for the infected patients (5). Precision for the colonized patients was set at 0.03. Precision was set at 0.05 for the infected patients considering the limitation of time and the rarity of the condition.

The calculation accordingly gave a value of 61 for the colonized group and 16 for the infected group of patients. Sample collection was extended to collect the maximum number of infected patients.

All patients admitted to the ICUs during the study period who gave voluntary informed consent and was not on antifungal treatment at the time of admission to ICU were enrolled in the study.

Patients who were already on antifungal treatment at the time of admission to ICU were excluded from the study.

Two adult patients were not included in the study due to severe injuries with pelvic and facial fractures as they were not stable to obtain screening cultures. Four extreme premature neonates were excluded from the study due to instability of their vital functions during handling.

Ethical approval was obtained from the Ethical Review Committee of the Faculty of Medicine, University of Ruhuna.

Informed written consent was obtained from patients

prior to enrolment for the study. Informed consent was obtained by the principal investigator after describing the purpose for the study and the procedure of specimen collection.

Patients were reassured of their anonymity and the confidentiality of data. Written voluntary informed consent was obtained from immediate family members available for adult patients who were unable to give consent due to their disease state and from the parents of Paediatric patients.

Statistical analysis

Data was collected using a data extraction sheet by the principal investigator. Medical details were extracted from the Bed Head Ticket and the past medical records available with patient. Data was analysed using SPSS version 20.

Results

Study sample included 152 consecutive admissions to the selected ICUs from 1st December 2015 to 30th March 2016.

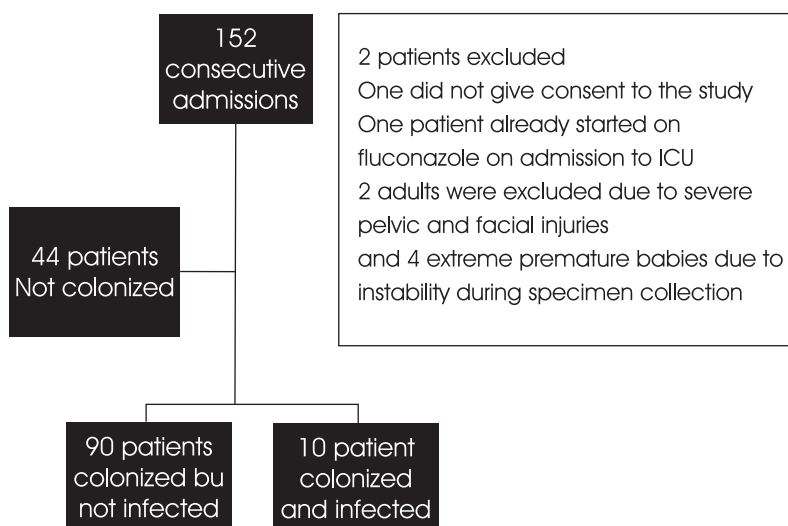


Figure 1: Sample composition

Candida colonization was found in 100 patients (65.7%) and only 10 patients of the sample developed blood stream *Candida* infection during the study period. The rest of the sample (90) was colonized with *Candida* but did not develop blood stream infection.

Age Categorization percentages

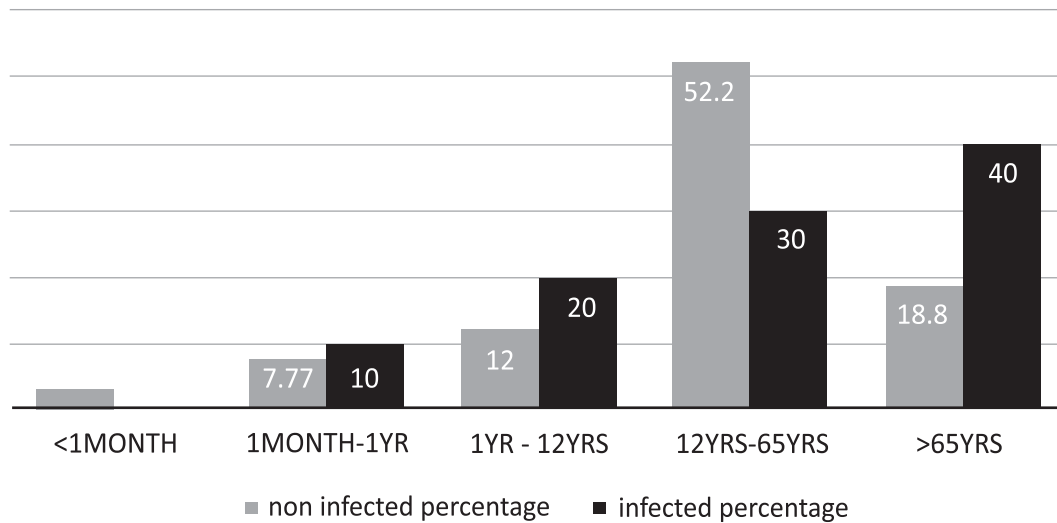
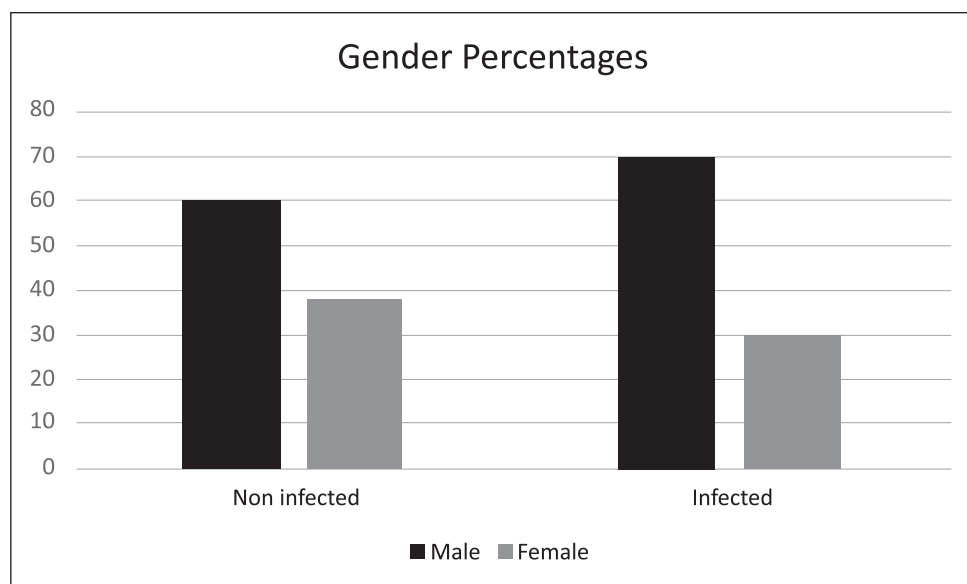


Figure 2: Age distribution of the sample in percentages

Most (40%) of the patients in the infected group were from more than 65 years age group and most (52.2%) of the non-infected patients were from the 12 years to 65 years age group. None of the infected patients were from the neonatal age group. However, one neonate who had heavy oral, rectal and urine colonization who had undergone surgery for omphalocele repair with sepsis was pre-emptively started on Fluconazole after discussing with the attending Physicians.



Distribution of patients in Neonatal ICU (NICU), Paediatric ICU (PICU), Main ICU, Emergency Treatment Unit ICU (ETC ICU) and Oncology ICU (Onco ICU) is shown in figure 4

Distribution in ICUs percentage

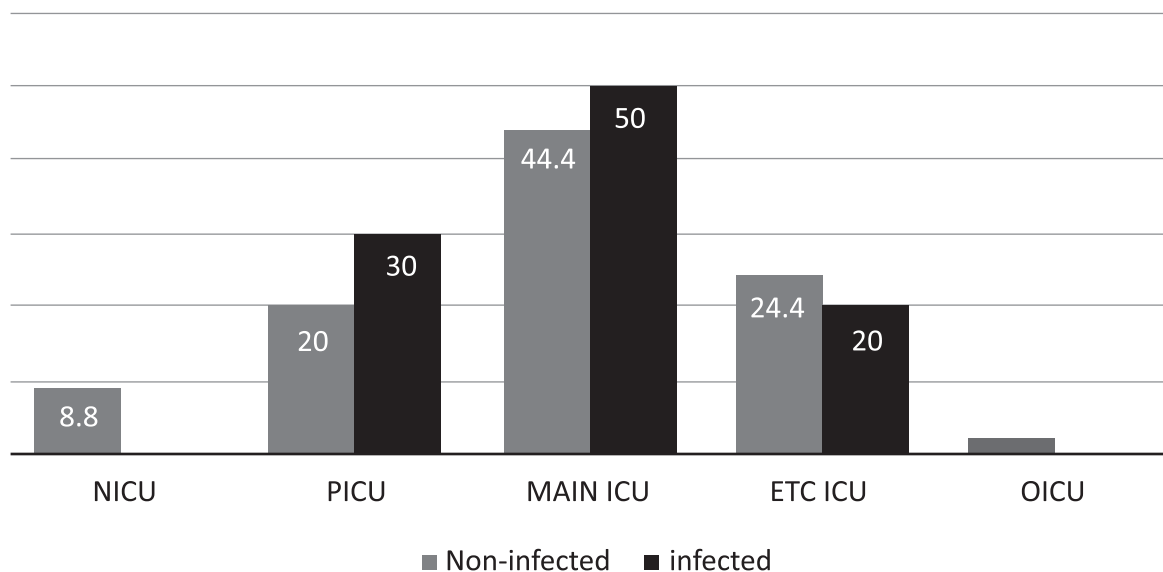


Figure 4: Sample distribution in the ICUs

Oral colonization was detected in 79 of non-infected patients while 66 non infected patients were rectally colonized. Urine colonization was detected in 18 of non-infected patients.

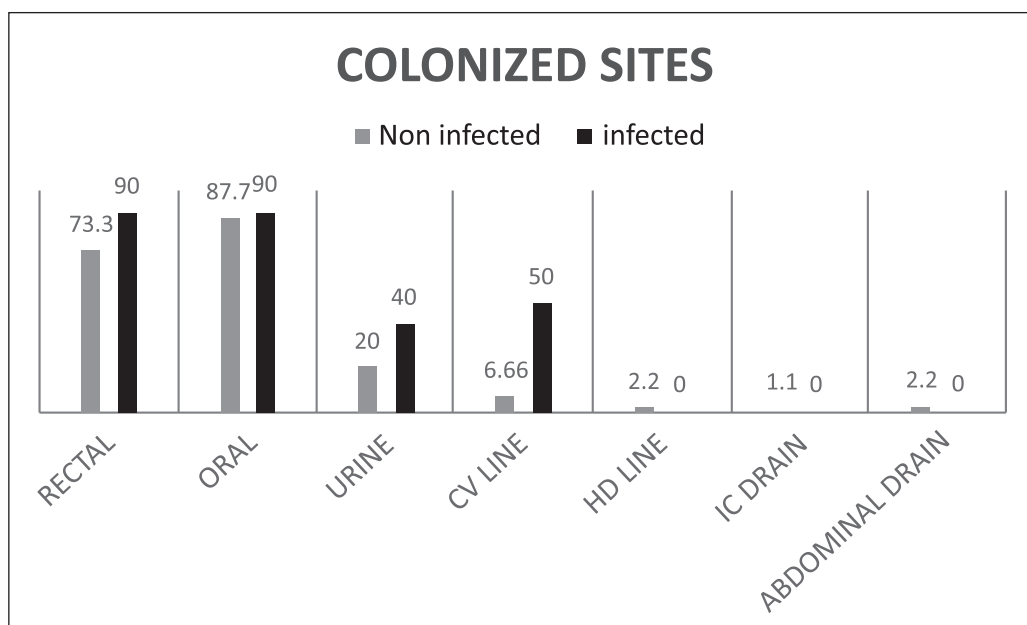


Figure 5: Percentage of patients colonized at screened sites

Patients were categorized as heavily colonized if they had more than 3+ growth in rectal and oral swabs and more than 105 Colony Forming Units/ml growth in urine cultures. Heavy colonization was seen only in rectum, oral cavity and urine. Other cultured sites did not have heavy colonization.

Comparison of heavy colonization among infected and non-infected population is given in figure 8.

Heavily Colonized Sites

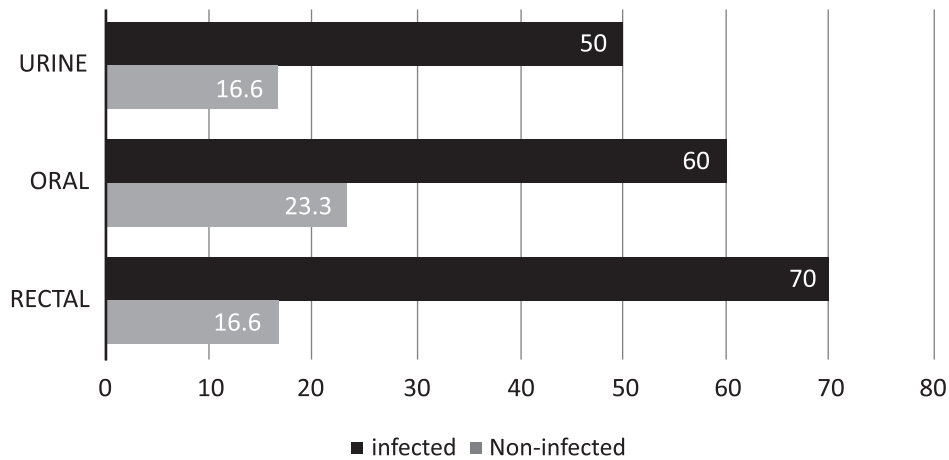


Figure 6: Comparison of heavy colonization in infected and non-infected patients

Candida colonization developed in the infected population at a mean of 1.4 ± 0.8 days. Maximum duration to develop colonization was 3 days.

Non-infected patients developed colonization at a mean of 1.78 ± 2 days. But the maximum duration to develop colonization was 12 days.

AFST was performed for each isolates except for repeating isolates of the same patient due to limitation of resources.

AFST results of non-infected patients are given in the table 2 with the average of sensitive zone diameter to Fluconazole, voriconazole and Amphotericin and the number of sensitive isolates for each species. Only the average zone diameter of the Amphotericin is given as there are no cut off diameters for disk diffusion method for Amphotericin according to the CLSI method.

	Total No	Fluconazole			Voriconazole			Ampho B Average diameter (Min-Max)
		S	I	R	S	I	R	
<i>C albicans</i>	82	78	2	2	80	1	1	25.63 (18-32)
<i>C tropicalis</i>	52	51	0	1	48	0	4	22.28 (15-31)
<i>C parapsilosis</i>	27	27	0	0	27	0	0	24.40 (15-38)
<i>C krusei</i>	13	-	-	13	12	1	0	20.61 (17-26)
<i>C glabrata</i>	8	0	5	3	7	1	0	20.12 (6-25)
<i>C famata</i>	1	1	0	0	1	0	0	22
<i>C kefyr</i>	1	1	0	0	1	0	0	26

Table 1: AFST of colonizing isolates of non-infected patients

(S – Sensitive, I – Intermediate, R – Resistant, Ampho B – Amphotericin B, Min – Minimum, Max- Maximum)

	Total No	Fluconazole			Voriconazole			Ampho B Average diameter (Min-Max)
		S	I	R	S	I	R	
<i>C albicans</i>	8	8	0	0	8	0	0	26 (24-28)
<i>C parapsilosis</i>	7	7	0	0	7	0	0	17.57 (6-25)
<i>C tropicalis</i>	6	6	0	0	6	0	0	21(15-26)
<i>C glabrata</i>	3	1	0	2	3	0	0	22.66 (21-24)

Table 2: AFST of colonizing isolates of infected patients

(S-Sensitive, I-Intermediate, R-Resistant, Ampho B-Amphotericin B, Min- Minimum, Max-Maximum)

All the colonizing isolates of *Candida albicans*, *Candida parapsilosis* and *Candida tropicalis* of infected patients were sensitive to both Fluconazole and Voriconazole. Only one of the three isolates of *Candida glabrata* was sensitive to Fluconazole but all of them were sensitive to Voriconazole

Table 3: Comparison of correlated factors of infected and non-infected patients
(p value less than 0.05 was considered significant)

Variable	Infected	Non infected	P value
Age (years)	46.36	38.51	0.390
Age >65	4	17	0.211
Male gender	7	55	0.583
CKD	0	4	0.000
DM	2	9	0.338
Congenital Heart Disease	1	8	1.000
Presence of CV line	8	56	0.323
CV line colonization with Candida	5	6	0.001
GI infection or surgical procedure	6	19	0.014
Surgery at ICU admission	4	30	0.731
Neurosurgical procedures	0	18	0.201
Disturbance of bowel integrity	4	14	0.077
Administration of TPN	1	9	1.000
Splenectomy	0	0	-
RTA	0	14	0.179
Immunosuppression	0	1	0.738
Chemotherapy	0	1	0.738

Presence of Sepsis, presence of a GI infection or surgical procedure, number of antibiotics given, CV line colonization and density of rectal, oral and urine colonization and change of colonizing species of *Candida* was significantly different among the two groups.

Sepsis was diagnosed by the clinician according to the Surviving sepsis campaign: International guideline for management of severe sepsis and septic shock 2012 (9)

All other factors had no significant difference.

The maximum recorded CI of the infected and non-infected patients during the screening period by the duration of colonization is given below in the scatter plot. Mean index of 0.5 was taken as the cut-off point similar to previous studies.

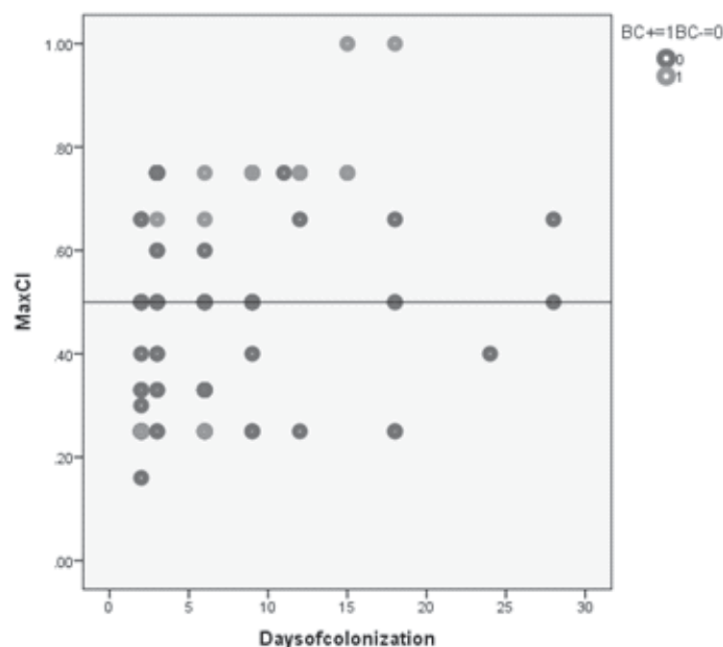


Figure 7: Scatter plot of Maximum CI with duration of stay (Green - infected patients, Blue – non-infected patients)

Maximum CI was reached in an average of 7 days in the infected group and 3.8 days in the non-infected group. Infection developed an average 5.6 days after reaching the maximum CI in the infected group of patients. Scatter plot of the Corrected CI with the duration of colonization is given. Cut-off point was set at 0.4 as in previous studies. However there were two patients who had values which were at zero as they didn't have heavy colonization.

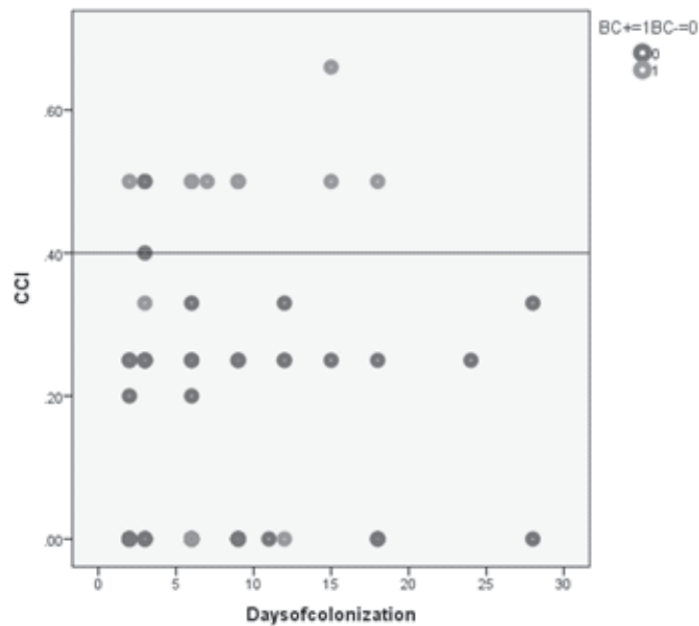


Figure 8: Scatter plot of CCI with duration of stay (Green - infected patients, Blue – non-infected patients)

Scatter plot for the Candida score with the duration of colonization is figure 13. The cut-off point for the Candida score was taken at the median value which was 3. All the infected patients had values more than or equal to a score of 3.

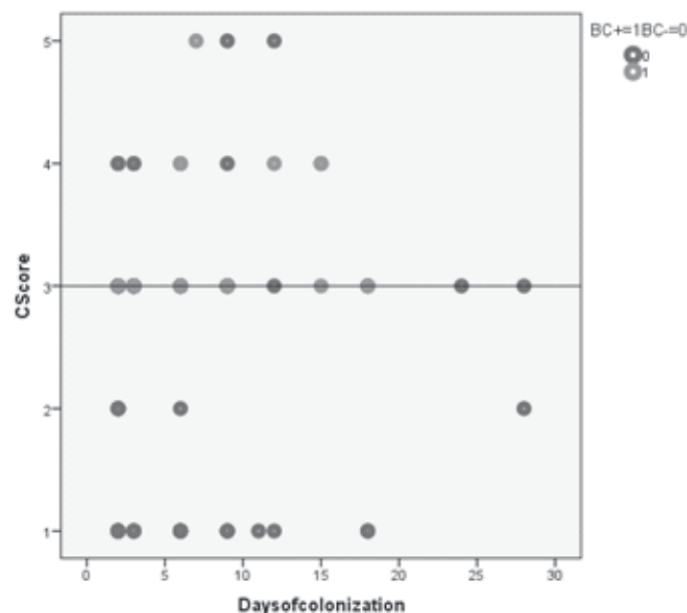


Figure 9: Scatter plot of Candida score with duration of stay (Green - infected patients, Blue – non-infected patients)

Therefore Maximum recorded CI of ≥ 0.5 , Corrected CI of ≥ 0.4 and a Candida score of ≥ 3 and New Candida score of ≥ 2 can be considered in the prediction of Candida blood stream infection.

The sensitivity, specificity, positive predictive value and negative predictive value are given in the table 5.

Parameter	Sensitivity	Specificity	PPV	NPV
MaxCI (=0.5)	80%	77.7%	28.5%	97.2%
MaxCI (=0.6)	80%	78.8%	32%	98.6%
Corrected CI	80%	94.4%	61.5%	97.7%
CS	100%	47.7%	17.5%	100%
CI at day 12	100%	76%	55.5%	100%
New CS (=2)	100%	63.3%	23.2%	100%
New CS (=3)	90%	87.7%	45%	98.75%

Table 4: Comparison of Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the tests CS- Candida score, CI-Colonization Index

Discussion

Ninety non infected patients and ten infected patients were included in the study. The minimum required sample of 16 infected patients was not achieved during the study period in spite continued enrolment of patients to satisfy the number while the non-infected patient population exceeded the required 61.

Considering the persistent heavy colonization and severe sepsis not responding to treatment with antibiotics as pre-emptive treatment, Fluconazole treatment was initiated in 9 patients in the non-infected group after discussing with the attending Clinicians and the Microbiologist.

Majority (62%) of the patients in the sample were male and in the 12 to 65 year age group. The main source of the study sample was from the main ICU and PICU which had a mixed population of surgical and medical patients who were critically ill.

Of the 144 patients studied, Candida colonization was found in 100 patients and 44 of the screened patients were not colonized. Therefore colonization percentage during ICU stay in this study sample was 69.4%. Colonization percentage of our population was similar to an Australian study done by Lau et al, (2015) on a mixed sample of medical and surgical patients and an Italian study conducted by Caggiano et al, (2011) on neurological patients admitted to

ICU(10,11).

Candida blood stream infection was seen in 6.9% of the total sample screened. A study done by the Extended Prevalence of Infection in ICU study group involving ICUs representing all regions of the world in 2011 showed that the point prevalence of Candidaemia is 6.9 per 1000 patients (0.69%) on the study day. Our rates showed a considerable increase from these rates compared to the countries studied. However this difference can be due to the small sample size in our study and the inability to include some patients who stayed for less than 24 hours who were admitted only for observation following surgery. This would have increased the sample number and would have in turn decreased the percentage of infected patients. Also the study was done as a point prevalence measure which is different from our study conducted over a period of 4 months.

Colonization was most frequent in the oral cavity (90%) and the rectum was colonized in all patients except one infected patient. Density of rectal, oral and urine colonization was significantly higher in the infected patients than non-infected patients. Therefore heavy colonization during ICU stay is a risk factor for Candidaemia. A study done in Switzerland on surgical patients had also described a significant increase in the density in infected compared to the non-infected patients (5).

Candida colonization was seen in 81% of the non-infected and 80% of the infected patients at admission and it increased to 100% with the duration of stay on the 9th day. This increasing trend was also described in the Italian study (11). But the Australian study done by Lau et al (2015) showed that colonized percentage increased from 60% at admission to 64% at 9 days (10). The difference in findings may be due to the larger sample of 6015 in the Australian study.

These findings show that prolonged duration of stay in ICU with continued exposure to risk factors increases the possibility of Candidaemia in these patients.

Colonization percentage reached 100% by the 9th day in the infected group and 12th day in the non-infected group. However there were 4 patients who never developed colonization up to 10 days and one patient who did not develop colonization up to 24 days of stay in the ICU. The infected group developed colonization at 1.4 ± 0.8 days which indicate that high risk patients should have been colonized on admission or the 3rd screening day. Therefore screening can be done on admission and repeated on the 3rd day and then weekly which will reduce the frequency of screening of patients.

However screening frequency was different in many studies. Pittet et al (1993) have done daily screening whereas Lau et al, (2015) did twice weekly and Leon et al, (2006) screened weekly (5, 10, 7).

US study done by Lau et al (2015) showed that most of the patients were colonized with Candida on admission and that few patients acquired colonization with the duration of ICU stay (10). This pattern is also seen in our study but a significant number of patients (80%) developed colonization during ICU stay. This may be due to the improved infection control practices observed in the USA as compared to TH Karapitiya.

A change of colonizing species of Candida during ICU stay was observed in 60% of the infected patients whereas it was only 15.5% in the non-infected group. The difference was statistically significant ($p=0.004$) and none of the previous studies had similar observations. This change could be due to replacement of the patient's own flora with the hospital flora or could be due to exposure of the patients to risk factors which may predispose them to develop this change. The interesting fact is that none of our patients were on

fluconazole during the specimen collection period. If they have been on fluconazole it could have been assumed that these isolates were replaced by resistant isolates. Further studies are required to identify the cause for this change which could be important in the management of these patients.

The fluconazole sensitivity for Candida albicans blood culture isolates were 100% and only one Candida glabrata isolate of the non albicans isolates was resistant to fluconazole.

The SENTRY surveillance study comparing the sensitivity of antifungals in all regions of the world show that Asia-Pacific and North American regions had 0.6% fluconazole and voriconazole resistance in Candida albicans whereas the European rate was 0.2% for fluconazole and voriconazole. Latin American region has 0% resistance to fluconazole in Candida albicans which is similar to the rates in our study. Fluconazole and voriconazole sensitivity for Candida glabrata was 13.5% in the Asia-Pacific region but the rate is lower (9.8% for Fluconazole and 11.1% for Voriconazole) in the North American region (12).

This SENTRY study shows the differences of antifungal susceptibility patterns in the different regions in the world and stress the importance of conducting studies specific to the region to identify the resistance patterns.

A similar AFST Pattern was seen in a study done in Spain which was less than 2% for Fluconazole in Candida albicans and less than 3.5% in Candida parapsilosis (13). The Latin American study done in 2013 show that 7% of the Candida glabrata isolates were fluconazole resistant and 92% of the isolates were only sensitive dose dependent (14).

In the non-infected population of our study, fluconazole sensitivity for colonizing isolates of Candida albicans was 95.5% and for voriconazole it was 97.7%. All the infected patients in our study were colonized with fluconazole sensitive Candida albicans isolates.

Of the 118 non albicans isolates subjected for AFST and 72% were sensitive to fluconazole while 94.9% was sensitive to voriconazole.

Candida glabrata infected patient in our study were colonized with the same species and showed similar

AFST pattern of fluconazole resistance. This isolate was sensitive to voriconazole.

Therefore identification of the colonization species and their sensitivity pattern is important to select the proper antifungal agent due to this high level of resistance to fluconazole in the colonizing isolates.

CV line colonization with *Candida*, presence of a GI surgery or infection, sepsis, number of antibiotics given and heavy colonization in rectum, oral cavity and urine were significant risk factors for the development of *Candida* blood stream infection in our study.

The NEMIS study done on Surgical ICU patients in 2001 showed that presence of a CV line, haemodialysis, undergoing surgery were significant risk factors for *Candidaemia*. Multisite *Candida* colonization was not found to be a significant risk factor in this study (15). This was a study done only on surgical patients although it was a multicentre large prospective study. This was the only study which described that multisite colonization was not a significant risk factor in the development of *Candidaemia*.

The study done by Leon et al (2006) showed that duration of stay in ICU, surgery at admission to ICU, total parenteral nutrition, haemodialysis, sepsis and multisite *Candida* colonization were significant risk factors for the development of *Candidaemia* (7). This study was conducted in 73 medical and surgical ICUs of Spain by the EPCAN project.

Administration of parenteral nutrition was a risk factor in both NEMIS study and the Spanish study (15, 7). This was not a significant risk factor in our study. The low numbers on parenteral nutrition in both infected (n=1) and non-infected (n=9) groups in our study could be the reason for this difference. Both studies were prospective multicentre studies conducted on a large number of patients. Therefore the low number of infected patients in our study could also be a reason for this deviation.

Haemodialysis was not a significant risk factor in our study. However it was found as a risk factor in both the above studies which were conducted on surgical and mixed populations (15, 7). None of the infected patients underwent haemodialysis and only 4 patients

of the non-infected group had a HD line and underwent haemodialysis. But two of the non-infected patients had *Candida* colonization in the HD line which was not found in the blood stream. Low numbers of dialysis patients is a limiting factor to study the effect of dialysis in our study.

Presence of a CV line was not a significant risk factor in the study done by Leon et al (2006) which is similar to our study (7). But the study done by Blumberg et al (2001) showed that presence of a CV line was a significant factor in the development of *Candidaemia* (15). However Blumberg et al (2001) conducted the study only on surgical patients whereas the study by Leon et al (2006) was conducted on mixed medical and surgical patients similar to our study sample which could be the reason for this similarity (15, 7).

Colonization of the CV line was a significant risk factor for the development of *Candidaemia* in our study. It was not found as a significant risk factor in other studies. However surgical procedures are considered to be risk factors in many studies including the above described two studies (15, 7). *Candida* colonization of the CV line is assumed to result from inefficient infection control practices (3). Therefore infection control practices should be strengthened in ICUs in the THK to prevent *Candidaemia*.

None of the antibiotics had a significant difference in the infected and non-infected group in our study. However, the study by Blumberg et al (2001) showed that antibiotics including vancomycin, carbapenems, β lactam- β lactamase inhibitor combinations and metronidazole had an increased risk of causing *Candidaemia* (15). The small numbers and the mixed medical and surgical patients in our study population as compared to large numbers and surgical patients could result in this deviation.

Chemotherapy and neutropaenia did not have a significant difference between the infected and non-infected groups. However the studied sample included only a very small number of patients on chemotherapy and neutropaenia.

A neurosurgical procedure was conducted on 18 of our patients in the non-infected group and none in the infected group. Neurosurgical procedures were not a significant risk factor for the development of *Candidaemia* in our study (p=0.201). However

patients who had neurosurgical procedures were significantly a lower risk for Candidaemia according to the NEMIS study (15). This is an important finding as there is a significant proportion of patients who are admitted to ICU following neurosurgical procedures and staying for a long a duration in ICU.

Maximum CI with a cut-off level of 0.6 identified all the infected patients except for two, one, a paediatric patient who refused specimen collection and the other who didn't have heavy colonization but had CV line associated Candidaemia.

Maximum CI with a cut-off level of 0.6 increased the specificity, positive predictive value and negative predictive value slightly.

Cut-off limit for corrected CI was taken as 0.4 similar to previous studies. The same two patients in the above Maximum CI fell below the cut-off level for Corrected CI. However the Corrected CI gave better specificity and positive predictive value than Maximum CI. Candida score had excellent sensitivity and negative predictive value in our study.

New Candida score was formulated by the risk factors identified in the current study for the sample of patients. This score gave excellent sensitivity and negative predictive values. However it was also not successful in increasing the positive predictive value for our sample.

Therefore, the combination of Candida Maximum CI, Corrected CI, Candida score, CI at day 12 and New Candida score improved the prediction of Candidaemia in our study.

Some patients who were pre-emptively treated with fluconazole were also included in the non-infected group of patients which was responsible for the reduction in the specificity and positive predictive value of this sample. However the low sensitivity of blood culture in detection of Candidaemia has to be considered. A study done in 2012 by the ESCMID study group showed that, if blood cultures were taken properly in a set of six for anaerobic and aerobic incubation, sensitivity of blood culture in detection of Candidaemia is 50 to 75% (16). This was not routinely done in our ICUs where only a single blood culture is taken at a time. The authors further recommend that the sensitivity can be improved up to 80% by

combining serial Mannan antigen and Anti-Mannan antibody detection. The advantage is that it will be positive in an average of 6 days prior to blood culture becoming positive. However cost and availability is a problem for the test.

Candida maximum CI, Corrected CI and candida score were all significantly different among infected and non-infected groups of patients ($p=0.000$, 0.000 & 0.02 respectively).

Similar studies on colonization index has taken a cut-off point of 0.5 for maximum CI and 0.4 for corrected CI. Sensitivity and specificity of the corrected CI was 100% in one of these earliest studies (5).

However results of studies done elsewhere have been variable as shown in the review done by the same authors who formulated the Candida colonization index (6). These studies showed that although sensitivity and negative predictive value in surgical patients is 100% it is 75% and 94% in the medical ICU respectively.

However, no study done up to date has been successful in the correct prediction of Candidaemia with 100% specificity and positive predictive value.

Candida score was formulated for non neutropaenic patients which makes it more suitable for our study sample as only two were neutropaenic. However, Candida score considered total parenteral nutrition, which is not a significant risk factor in our study. Therefore the usage of Candida score with total parenteral nutrition for our sample is questionable. However Candida score gave the best sensitivity and negative predictive value for our sample of patients. A prospective multi-centre study done by Leon et al in 2009 in Spain, Argentina and France also showed a significant negative predictive value of the Candida score. However the specificity was 66.2% for the Candida score. Invasive infection was not reported in patients with a Candida score below 3 (17).

Ahmed et al (2014) in their review described that models combining clinical as well as Microbiological parameters are better in predicting Candidaemia (18). The authors further described that a risk prediction model can vary due to the effects of geographic location, time, and variations in antibiotic prescription and the virulence of the colonizing Candida species. This review stressed the importance of a prediction

rule customized for the population which should be reviewed appropriately.

Conclusions

Candida score and the New Candida score formulated for the study sample has excellent sensitivity with poor specificity in prediction of Candidaemia. Combination of Candida score with the Corrected Colonization Index which had good specificity can reliably predict Candidaemia in our study sample.

Presence of Sepsis, presence of a GI infection or surgical procedure, number of antibiotics given, CV line colonization and density of rectal, oral and urine colonization and change of colonizing species of Candida were identified as risk factors for Candidaemia for the sample.

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Ongoing postgraduate research projects registered at the Board of Study in Medicine, Faculty of Medicine, University of Ruhuna & Faculty of Graduate Studies, University of Ruhuna.

MPhil Studies

1. **Development and use of a new instrument to evaluate parenting competencies among mothers with infants at early infancy in Galle District**

Candidate- A.D.S.S. Karunanayaka, Department of Nursing, Faculty of Allied Health Sciences, University of Ruhuna

Principal Supervisor- Dr. C.J. Wijesinghe, Senior Lecturer Department of Community Medicine, Faculty of Medicine, University of Ruhuna

Co-supervisor: - Prof. K.G. Somasiri, Associate Professor, Department of Physiology, Faculty of Medicine, University of Ruhuna

Funding: Faculty of Medicine Research Grant, University of Ruhuna

2. **Effects on health and social wellbeing due to drug abuse among institutionalized male drug addicts in Four Districts, Sri Lanka**

Candidate - Dr. I L A N Darshana, Lecturer in Community Medicine, Department of Community Medicine, Faculty of Medicine, University of Ruhuna

Supervisors - Prof. P V De Silva, Professor and Consultant Community Physician, Department of Community Medicine, Faculty of Medicine, University of Ruhuna

Dr. C J Wijesinghe, Senior Lecturer and Consultant Community Physician, Department of Community Medicine, Faculty of Medicine, University of Ruhuna

Funding: UGC Grant (UGC / VC / DRIC / PG 2016 (II) / RUH / 01)

3. **Age related trends and population specific reference ranges of bone mineral density and bio markers of bone turnover in adult females**

Candidate - Mrs. Hasanga Rathnayake, Lecturer (Probationary), Department of Biochemistry, Faculty of Medicine, University of Ruhuna

Supervisors - Prof. Sarath Lekamwasam Professor of Medicine, Department of Medicine, Faculty of Medicine, University of Ruhuna.

Dr. C.M. Wickramatilake, Senior Lecturer, Department of Biochemistry, Faculty of Medicine, University of Ruhuna.

Prof. Janaka Lenora, Department of Physiology, Faculty of Medicine, University of Ruhuna.

Funding: UGC Block Grant

4. Colonization and in-hospital transmission patterns of methicillin-resistant *Staphylococcus aureus* among surgical and medical patients at Teaching Hospital Karapitiya

Candidate - Ms. MRP Kurukulasooriya, Duke-Ruhuna Research Centre, Faculty of Medicine, University of Ruhuna

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Dr. WMDGB Wijayaratne, Department of Microbiology, Faculty of Medicine, University of Ruhuna

Prof. CK Bodinayake, Department of Medicine, Faculty of Medicine, University of Ruhuna

Dr. LG Tillekeratne, Duke University, USA

Prof. CW Woods, Duke University, USA

Prof. Truls Ostbye, Duke University, USA

Dr. Dharshan de Silva, Faculty of Medicine, Kotelawala Defense University

Funding - UGC Block Grant (RU/PG-R/16/03)

5. Validation of Mississippi Aphasia Screening Test and the effect of aphasia on quality of life of patients with stroke

Candidate - Ms. Kariyawasam Gamage Pramudika Nirmani, Lecturer, Department of Nursing, Faculty of Allied Health Sciences, University of Ruhuna

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Dr. D. C. Hewage, Department of Physiology, Faculty of Medical Sciences, University of Sri Jayewardenepura

Funding - Grant from research and higher degrees, Faculty of Medicine, University of Ruhuna

6. Vasculopathy, systemic inflammation, body composition and cardiometabolic risk among patients with chronic kidney disease

Candidate - Mrs. E. H. Silva, Lecturer (probationary), Department of Medical Laboratory Science, Faculty of Allied Health Sciences, University of Ruhuna

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Prof. S. Lekamwasam, Professor of Medicine, Department of Medicine, Faculty of Medicine, University of Ruhuna

Prof. L.K.B. Mudduwa, Department of Pathology, Faculty of Medicine, University of Ruhuna

Dr. R. A. Ubayasiri, Consultant Vascular and Transplant Surgeon, Vascular and Transplant Unit, Teaching Hospital, Karapitiya

Funding - UGC block grant (Grant Number : RU/PG/16/06)

7. **Protective effects of selected medicinal plant extracts in rats with chemically induced nephropathy**

Candidate - Mrs. A.M.S.S Amarasiri, Lecturer (Probationary), Dept. of Medical Laboratory Science, Faculty of Allied Health Sciences, University of Ruhuna.

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Prof L.K.B. Mudduwa, Professor of Pathology, Department of Pathology, Faculty of Medicine, University of Ruhuna

Funding - NSF Competitive Research Grant and UGC Block Grant for Strengthening Research

8. **Effect of a standardized herbal drug of *Coccinia grandis* (L.) Voigt on metabolic profile in patients with type 2 diabetic mellitus**

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Funding - National Research Council (Grant no. 17-029)

PhD Studies

1. **Effects of metformin and lifestyle modification on the progression of atherosclerosis among individuals with impaired glucose tolerance**

Candidate - Dr. A.T.I.M.Amarasinghe, Lecturer (Probationary), Department of Pharmacology, Faculty of Medicine, University of Ruhuna

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Funding- University Grants Commission:- 2016–UGC/2015/RUH/01

2. **Anthracycline induced cardiotoxicity; A clinical, ethnopharmacological and biochemical approach**

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Funding - University Grant Commission (UGC) research grant; (UGC/VC/DRIC/PG2015(III)/RUH/01)

University of Ruhuna research grant (2015/RU/PG-R/15/03)

Prescribing Aminoglycosides – practicing correctly

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Abstract

Aminoglycosides are still a commonly used first-line group of drugs in the treatment of life threatening infections due to their rapid effectiveness and lack of diffogenicity. They are often used in surgical prophylaxis, and in empirical therapy of neonatal infections.

Despite very clear guidelines, aminoglycoside dosing and prescription are often carried out incorrectly, which can cause serious dose-related side effects including nephrotoxicity and irreversible hearing loss, emphasizing the importance of ensuring that the patients receive the correct dose and are monitored regularly to improve the safety and efficacy of the drug.

This review summarizes why aminoglycosides are still a valid treatment option and discusses the rationale for current dosing regimens in Gram-negative infections. In particular it focuses on the various methods for monitoring aminoglycosides that are currently being used.

Introduction

Since the introduction of aminoglycosides into clinical practice, several other groups of antibiotics have come to the market. However, the increasing prevalence of infections due to Gram-negative bacterial strains with advanced patterns of antimicrobial resistance, has prompted physicians to keep relying on this 'old' group of antibacterials.

Unlike most of the other antibiotics which predispose to gut colonization and infection with pathogenic bacteria including *Clostridium difficile*, according to Qureshi I et al, 2012, gentamicin is very much less likely to lead to antibiotic associated diarrhea and colitis due to *C. difficile*. Styrt B, Gorbach SL, 1989 have excluded vancomycin and parenterally administered aminoglycosides as antibiotics associated with pseudomembranous colitis and diarrhoea.

Uses of aminoglycosides and the spectrum of antimicrobial activity

Aminoglycosides have a broad spectrum of activity with more gram negative cover. However, gentamicin is used in combination with beta-lactams for the synergistic action against gram positive bacteria. This group of drugs do not have an anaerobic cover. Examples of clinically encountered bacteria exhibiting endogenous resistance include *Stenotrophomonas maltophilia* and *Burkholderia cepacia*, two uncommon but emerging pathogens in health care environment. Bacterial infections in which they are commonly used include, osteomyelitis, endocarditis, intra-abdominal infections, pelvic inflammatory disease, meningitis, urinary tract infections, sepsis and neonatal infections (Gilbert DN, Leggett JE, 2010).

Gentamicin is also recommended in surgical prophylaxis (Bratzler DW et al, 2013).

Efficacy and safety of gentamicin in the treatment of neonatal infections was proven as early as 1970s when it was first introduced into clinical practice (McCracken, GH, 1970). Even today, its value in empirical therapy regimen for late-onset neonatal sepsis in health care settings is proven, especially where the prevalence of beta lactam resistance is high (Weerasinghe NP et al, 2018).

Safety of gentamicin in pregnancy and breast feeding

Aminoglycosides may cause auditory or vestibular nerve damage in infants if used during the second or third trimesters of pregnancy, and should be avoided if possible. If treatment is required, gentamicin is the preferred aminoglycoside because, although it does cross the placenta, it has not been associated with teratogenic risk with regard to structural development of the fetus (Czeizel AE et al, 2000)

Drugs and Lactation database of US National Library of Medicine states that gentamicin and amikacin, the

most commonly used aminoglycosides, can be used safely with multiple daily dose regimens in patients who are breastfeeding.

Pharmacokinetics/ dynamics of gentamicin

They are not absorbed from the gastrointestinal tract when administered orally, but are rapidly absorbed after intramuscular or subcutaneous injection. They can also be given intravenously, intrathecally or as topical preparations. Other routes of administration include inhalation through a nebulizer (tobramycin), intraperitoneal and intraventricular administration (gentamicin). Neomycin is used orally for their bowel intra-luminal activity, as it is not systemically absorbed.

It crosses the placenta, but very poorly crosses the blood-brain barrier into the central nervous system (even when the meninges are inflamed) or penetrate the eye. Tissue levels are low except in the renal cortex. The plasma half-life is 2-3 hours. Gentamicin is eliminated unchanged, entirely by glomerular filtration (Gilbert DN, Leggett JE, 2010)

Mechanism of action

Aminoglycosides act by irreversibly binding to 30S subunit of the bacterial ribosome, negatively impacting on protein synthesis. The binding interferes with the initiation complex and misreading of mRNA leading to incorrect amino acids being inserted into the polypeptide chain producing nonfunctional or toxic peptides (Greenwood D, Ogilvie MM, 2012).

Aminoglycosides; amikacin and gentamicin are drugs displaying concentration dependent killing, with increasing levels of drug resulting in increasing bactericidal action. Concentration-dependent post antibiotic effect (PAE), in which bactericidal action continues for a period of time after the antibiotic level falls below the minimum inhibitory concentration (MIC), peak concentration and area under the concentration curve (AUC) determine the efficacy of gentamicin in vivo (Levison ME. 2004; Wallace AW, 2002)

Unwanted effects of aminoglycosides

Nephrotoxicity is a well-known side effect of all the aminoglycosides. In some cases, the problem is so severe the use of the drug has to be discontinued. It has

been estimated that up to 30% of patients treated with aminoglycosides for more than 7 days show some signs of nephrotoxicity (Mathew, 1992). Other adverse effects can be less severe reactions, such as nausea and vomiting, or any other more severe reactions including, low blood cell counts, allergic reactions, neuromuscular problems, nerve damage (neuropathy) and ototoxicity.

Nephrotoxicity and ototoxicity are thought to be dose related with higher doses causing greater chance of toxicity.

Gentamicin accumulates in proximal renal tubular cells and causes cell damage. Tubular cell regeneration occurs despite continued drug exposure. Therefore in 5 - 25% of patients mild and reversible nephrotoxicity may be observed. Excretion of gentamicin almost totally dependent on kidneys, leading to a vicious cycle of increasing drug levels with renal failure, in the body.

Ototoxicity due to gentamicin is irreversible. It appears to be correlated to cumulative lifetime exposure. However, ototoxicity may appear just after a single dose of the drug or can have delayed presentation, sometimes not appearing until after completing treatment (Mudd PA, 2018). Further toxicity may lead to degeneration of the 8th cranial (vestibulocochlear) nerve- features include hearing loss, vertigo, nausea, vomiting, dizziness and loss of balance.

Incorrectly overdosing the patients with gentamicin can accelerate the occurrence of above adverse drug events and prescribing errors are not uncommon in healthcare settings. A study by Lesar TS et al, 1997, have found that the commonest factor associated with medication errors, non-alteration of drug therapy with the decline in hepatic or renal function (13.9%) and 11% with incorrect dosage calculations.

Anecdotally, incorrect under dosing is also a problem, since the lower doses lead to poor clinical improvement and therefore prolonged courses of antibiotics, which can increase patient morbidity and development of drug resistance in bacteria in the face of low levels of antimicrobials.

Not only the dose of the drug, but also the dosing frequency is important, since impaired excretion can lead to drug accumulation in the body leading to

toxicity.

An audit assessing the possibility of implementing the online gentamicin dose calculator and gentamicin serum level monitoring using Urban and Craig nomogram replacing a modified nomogram in a hospital under National Health Services (NHS) England, has found that 56% of initial gentamicin dose calculations were incorrect before implementing the calculator and 14% of the serum gentamicin levels were falling out of the safe range at a given time post dose with the modified nomogram (Weerasinghe NP, 2017).

Therefore, the value of aminoglycosides in clinical practice would be greatly enhanced if some means could be found to overcome the associated dosing errors.

The question therefore arises how the Sri Lankan healthcare could improve the practice of prescribing aminoglycosides within the resource poor settings. In this review we shall briefly cover this issue, highlighting the current evidence for and against the practice of prescribing aminoglycosides.

How we could safely prescribe gentamicin

Under the umbrella term of 'antibiotic stewardship' correct indication for starting antimicrobials, correct selection of antimicrobial agents and correct dosing regimens are all important, to protect the antimicrobial agent as well as the patient.

Considering the patient safety, several factors have been identified to increase the risk of aminoglycoside associated nephrotoxicity that cannot be modified readily by the clinician (e.g., sex, obesity, preexisting liver or renal diseases and underlying diseases) and factors that the clinician may be able to modify (drug dosage/interval/duration, specific aminoglycoside, hypokalaemia, hypomagnesaemia, metabolic acidosis, volume depletion and concurrent medications) (Kaloyanides, 1993).

Correct indication for starting antimicrobials

Presence of bacterial infection should be confirmed either clinically or by laboratory findings. National guidelines for empirical and prophylactic use of antimicrobials, 2016 have recommended aminoglycosides; gentamicin, amikacin etc. for infections due to common pathogenic gram negative

bacteria, *Escherichia coli* and other coliforms, *Pseudomonas* and some *Proteus* species, *Staphylococci* and other gram-positive organisms. Many aminoglycosides are available in parenteral formulations (especially as intravenous preparations) and administering them to a patient is an invasive procedure. Keeping an intravenous cannula for several days than recommended may lead to cannula site infections due to the breach in infection control measures. Therefore before prescribing an aminoglycoside, adequate risk benefit assessment should be done. When the cannula is in place, the patient also needs to be daily assessed for the necessity of continuing the drug

Correct selection of patients

Individual patient factors like patient's age, body weight, pregnancy status, renal function, liver function, other medications the patient is currently on as well as the cost of the drug, availability, side effects and toxicities associated with the drug also needed to be taken into consideration.

Elderly patients are especially vulnerable for the toxicity associated with aminoglycoside therapy. Patterson DL, et al 1998, states that the risk of both nephrotoxicity and ototoxicity was more likely to be associated with longer duration of gentamicin therapy in them. On the other hand, elevated baseline serum creatinine levels in the elderly has been found to be associated with renal toxicity alone. They also found that limiting the duration of aminoglycoside therapy to less than a week could substantially reduce the risk of toxicity.

Concurrent medications that should be taken into consideration

Aminoglycosides should not be started in patients who are already on drugs that cause nephrotoxicity (e.g. cyclosporine, amphotericin B and allopurinol) or ototoxicity (e.g. furosemide) (Garrahan F, Fallon R, 2015; Patterson DL et al 1998)

In patients with myasthenia gravis, gentamicin is contraindicated because of its tendency to cause neurotoxicity- clinically significant muscle weakness due to impairment of neuromuscular transmission (NHS Greater Glasgow & Clyde Area Drug and Therapeutics Committee).

How to calculate the correct dose of aminoglycoside (gentamicin and amikacin) and monitor patients to prevent toxicity.

These are among the few antibiotics which are weight-dependent in adults. Ideal body weight (IBW) should be used in all non-obese patients, unless actual body weight is lower; in these patients actual body weight should be used. IBW is calculated according to the sex and the height of the patient.

IBW Male (kg) = $50 + (2.3 \times \text{number of inches above five feet in height})$

IBW Female (kg) = $45.4 + (2.3 \times \text{number of inches above five feet in height})$

Aminoglycosides are highly hydrophilic and are not distributed into adipose tissue. Therefore in obese patients corrected body weight (CBW) should be used for dosing calculations rather than IBW. Otherwise they can get overdosed if actual body weight (ABW) is used. CBW is calculated using ABW and IBW of the patient.

$$\text{CBW} = \text{IBW} + 0.4 (\text{ABW} - \text{IBW})$$

Guessing the patient's weight, which happens most of the time leading to severe under dosing or overdosing, will therefore be avoided. Gentamicin dose calculator which is available online removes the extra burden of calculating the dose manually.

Other aminoglycosides (e.g. amikacin, streptomycin and tobramycin) can be dosed using similar methods, but use different dose-weight ratios.

Patient's renal function also matters when calculating the dose. Assessment of renal function is done by measuring estimated glomerular filtration rate (eGFR) or more accurately creatinine clearance.

Gentamicin is given mainly using two regimens-extended interval regimen (eg. once daily dosing) and multiple daily dosing. Due to the concentration dependent nature of the drug, once daily regimen is practiced to get the optimum bactericidal effect in most of the infections, Since the level of bactericidal activity is based on the peak and the toxicity correlates with high troughs, giving one large dose leading to a high peak and less total time above the recommended trough level as compared with divided doses, will reduce toxicity without compromising clinical

efficacy.

Long enough dosing interval also helps impeding the development of bacterial resistance to drug uptake, thereby protecting the efficacy of the drug (Craig W, 1998).

However, for infective endocarditis, where we need gentamicin to act synergistically with beta-lactam antibiotic, three times per day regimen is used. Lack of evidence does not warrant once daily dosing of gentamicin in pregnant mothers, in neonates and in paediatric infections as well. Patients with burns of more than 20% of the total body surface area and those with a creatinine clearance of less than 20ml/min are also excluded from this regimen (Banerjee S, et al. 2012)

Once daily regimen uses an initial dose of intravenous gentamicin of 5 - 7mg/kg, unless there is renal insufficiency, in which a reduced dose (e.g. 2–3mg/kg) should be used. Amikacin is given as 15mg/kg body weight with normal renal functions. Therefore, baseline creatinine clearance should be checked for all patients. Using lower doses of gentamicin or amikacin in patients with renal impairment to reduce toxicity has no ill effect on the efficacy of treatment, since the impaired excretion of the drug will lead to adequate drug levels in the body. The frequency of dosing and timing of the next dose depends on the patient's renal function. Therefore monitoring of aminoglycoside therapy is essential to ensure optimal treatment and to prevent toxicity (Banerjee S, et al. 2012).

Aminoglycoside monitoring

This is achieved in two ways- monitoring aminoglycoside concentration in blood, and monitoring the patient for potential adverse reactions. Aminoglycoside level monitoring can be done by several methods- trough level only, peak and trough level measurement and using nomograms.

Renal functions should be measured at the start of the treatment and every 2 – 3 days thereafter (more frequently if necessary). For patients with altered volume of distribution or creatinine clearance, monitoring frequency should be individualized as appropriate and expert help sought.

If the treatment is going to be more than 72 hours, patients should be informed about potential

ototoxicity and assessed at the beginning and weekly thereafter.

Monitoring trough level only

If renal function is adequate ($\text{Cr Cl} > 60 \text{ ml/min}$) and trough level of the drug (taken within an hour before the second dose) is $< 1 \text{ mg/L}$, the next dose of gentamicin can be given at 24 hours from the previous dose. If the trough level is $> 1 \text{ mg/L}$, extending the dosing interval is recommended. If the renal function is also impaired, more frequent monitoring and seeking expert opinion would be helpful. This will need good coordination between medical and paramedical staff- biochemist, microbiologist, clinician and pharmacist.

Nomograms

Nomograms can be used to monitor aminoglycoside therapy in extended interval dosing. Hartford nomogram and Urban and Craig nomogram are commonly used for gentamicin dose of 7 mg/kg and 5 mg/kg dosing regimens respectively. (Figure 1 & 2)

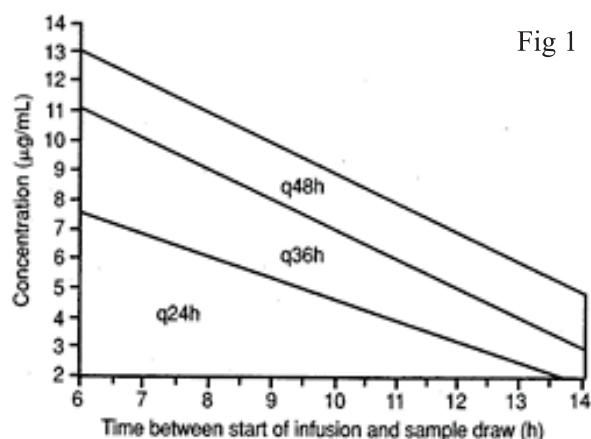


Fig 1

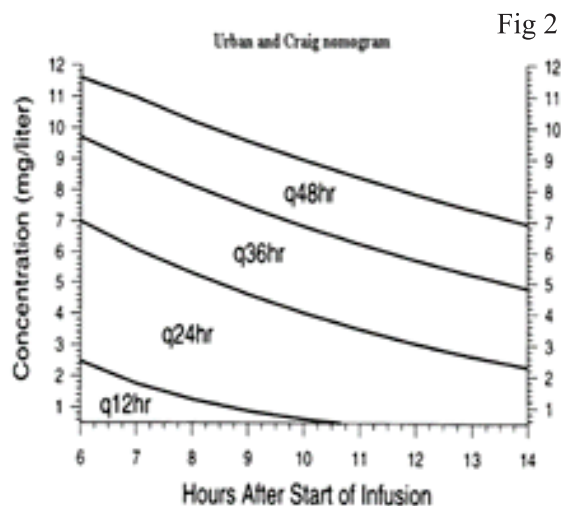


Fig 2

Nomograms act as a guide on when to give the patient their next dose based on gentamicin concentration and the time, the measurement was taken.

Multiple dose regimen

In patients with normal renal function, measure aminoglycoside level after the first or second maintenance dose (Gilbert DN, Leggett JE, 2010). To monitor multiple dose regimen, measure serum peak level 30 minutes after completion of the intravenous infusion or one hour after the intramuscular injection and measure trough level immediately before the next scheduled dose (Brunton LL et al, 2010). If the trough level is within the expected range monitor renal function every three to five days and, while the renal function remains stable, further aminoglycoside monitoring is not necessary. If renal function deteriorates, assess the patient, and if it is necessary to continue gentamicin, recalculate the dose and measure the serum level after starting the new regimen (Gilbert DN, Leggett JE, 2010). If the trough level is high, omit the next dose and measure the level for the expected trough level. Assess the situation on an individual basis and adjust the dose and duration.

Conclusions

Instituting these interventions can have definite positive impact on improving aminoglycoside prescription, ensuring safer care for the patients, our ultimate goal. Audits will be needed to assess the impact of these changes over the time, especially the improvement of clinical outcomes.

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Cleft Palate: Genetic screening and syndromic diagnosis – a multi-disciplinary approach in the management

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Introduction

History

Since the ancient times man was ignorant of embryology and morphogenesis, his explanation for the existence of congenital deformities was based on a combination of religion, superstition, invention and charlatanism. Congenital deformities, including the cleft lip and palate, were considered to be evidence of the presence of an evil spirit in the affected child. Facial deformities were most condemned and the infants were “removed from the tribe or cultural unit and left to die in the surrounding wilderness”, a practice that still prevails today in certain African tribes. Fabricius ab Aquapendente (1537–1619) was the first to suggest the embryological basis of these facial clefts. He suggested that in the development of the human fetus, the upper lip only coalesces along the middle line at a very late stage. However, the most convincing explanation of the origin of the facial cleft in this period was furnished by Philippe Frederick Blandin (1838–96), who suggested that it resulted from a failure of the premaxilla and the maxillary segments to unite (1,2). A successful operation on a cleft palate however did not occur until 1816. Graefe in 1816 and Roux in 1819 published the first satisfactory results. After the introduction of chloroform cleft surgery made remarkable progress (2).

Cleft palate

Cleft palate (CP) is a congenital fissure in the roof of the mouth, resulting from incomplete fusion of the palate during embryonic development (3). Extreme variability can be seen from a submucous cleft to a cleft involving the whole of the soft and hard palate. Cleft palate is one of the commonest congenital malformations of the head and neck region and can be associated with cleft lip and other congenital anomalies. Orofacial clefting occurs in all races, both sexes and all socio-economic groups (4). Untreated or incorrectly treated oro-facial clefts contribute

substantially to long-term disability in children, as well as tremendous emotional and financial stress for affected families and individuals (4). The treatment is a long term process and starts soon after the birth and continues to the end of the second decade of life. It requires a multidisciplinary approach with feeding and respiratory assessments, surgery for repair of the defect (this may sometimes involve multiple procedures), speech therapy, orthodontic management, autolaryngological and audiological assessment and management and, pediatric follow-up (4).

The reported incidence of cleft lip and palate has been increasing across the world. In a study from Denmark, reported that, the incidence of cleft lip and palate had doubled during the last fifty years and tripled during the last one hundred years. In a twenty eight year follow up study reported from Finland, showed a rapid increase in incidence of cleft lip and palate (5,6).

Development of Palate

Early development of the face occurs between the fourth and eighth weeks of gestation and is dominated by proliferation and migration of the neural crest ectomesenchyme. The face develops from one median frontonasal, two maxillary and two mandibular prominences around the stomodeum (3,7).

The primary palate is a wedge shaped mass of mesenchyme between the internal surfaces of the maxillary prominences of the developing maxillae. It forms the premaxillary part of the maxilla and is represented in the adult hard palate as the area anterior to the incisive foramen bearing the incisor teeth (3,7,8). The main part of the definitive palate is formed by two outgrowths of maxillary prominences. Palatal development occurs between the sixth and eleventh weeks of intrauterine life. The palatine shelves grow from the inner aspect of the maxillary processes. At first, they hang vertically downwards with the tongue between them. As the neck begin to extend between eighth and ninth week of gestation,

the tongue moves downwards and the palatal shelves become horizontal by an active process (3,7,8). The elevated palatal shelves fuse with the primary palate anteriorly and with each other more posteriorly. The mesoderm of the palate undergoes intramembranous ossification at around 8 weeks of gestation to form the hard palate except in the posterior most region where it remains unossified to form the soft palate and it involves a process of epithelial cell adhesion and fusion followed by epithelial seam disintegration and mesenchymal ingrowth. The mistiming of any of these essential events, due to an environmental factor or genetic predisposition, results in the failure of fusion, leading to clefts of the palate (3,7,8)

Prevalence of Cleft palate

The prevalence of the cleft lip with or without cleft palate and isolated cleft palate varies in different populations with an overall incidence of oral clefts ranging from 1 in 750 live births in Asians to 1 in 2500 live birth in African Americans (9). The incidence of cleft lip with or without cleft palate varies with race. The highest reported incidence was in American Indians, at 3.6 per 1000 live births and the lowest incidence among African Americans, at 0.3 per 1000 live births (9,10).

In Europe, the highest incidence of cleft lip with or without cleft palate is in the Netherlands (1.46 in 1000 live births) and the lowest in France (0.67 in 1000 live births) (11). The highest incidence of isolated cleft palate was found in Finland (0.97 in 1000 newborn child) and the lowest in Denmark (0.36 in 1000 newborn child) showing marked difference in incidence of cleft palate in different ethnic and racial groups (11,12).

In Sri Lanka, data is available from the Central province using data from a tertiary care centre reported the incidence of cleft lip and palate to be 0.83 per 1000 live births, while the incidence of isolated cleft palate was 0.19 per 1000 live births. Family history of oral clefting was found in 9.1% of the subjects in this population (13). A study of congenital malformations among all live births at the General Hospital, Anuradhapura, over one year period from February 2002 to January 2003 found that the prevalence of cleft lip with or without cleft palate was 2.19 per 1000 live births (14).

Etiology of Cleft palate

There are several causative factors that have been recognized in the aetiology of cleft palate (7,8). Disruption of the palatal mesenchyme affects palatal epithelial cell proliferation leading to failure in palatal shelves' adhesion and fusion and is one of the commonest factors associated with cleft palate. Abnormalities in palatal shelf movements with growth of the tongue can lead to widening of the gap between the two palatine shelves and failure of meeting in the midline. Cleft palate that is seen in Pierre Robin sequence is an example for this type of failure (7,8).

There are several genetic and environmental factors involved in the process of morphogenesis of the primary and secondary palates. Shifting of this complex process toward a threshold of abnormality by adverse genetic and environmental conditions, can initiate the process of clefting of the lip and/ or palate (15,16).

Genetic studies on human populations have demonstrated that non syndromic cleft lip with or without cleft palate (CL+/-P) and isolated cleft palate (ICP) have distinct genetic backgrounds (8,17).

Associated anomalies

Teratogenic and genetic insults to the process of embryogenesis result in malformations. The type and extent of the malformation would depend on the developmental stage of the embryo at the time of insult. The period of vulnerability to the induction of malformations of a specific organ system is referred to as the critical period (18). Patients with oral clefts may also exhibit other anomalies. However, the data is conflicting with widely different estimates for each associated anomaly varying between 2-65% in different countries. The associated anomalies include those affecting the cardiovascular, musculoskeletal, genito-urinary and gastrointestinal systems. Psychological problems are also seen more frequently among cleft palate subjects (19-23).

Table 1 Common syndromes associated with cleft lip and palate (8).

Syndrome	Gene involved
Stickler	COL2A1 (Collagen, type II, alpha 1)
Apert	FGFR2 (Fibroblast growth factor receptor 2)
Otopalatodigital	FLNA (Filamin A, alpha)
Pallister Hall	GLI3 (GLI family zinc finger 3)
Kabuki	MLL2 (Myeloid/lymphoid or mixed-lineage leukemia 2)
Cornelia de Lange	NIPBL (Nipped-B homolog)
Oro facio digital	OFD1 (oral-facial-digital syndrome 1)
Campomelic dysplasia	SOX9 (SRY (sex determining region Y)-box 9)
DiGeorge	22q11 deletion including <i>TBX1</i> (T-box 1)
Treacher Collins	TCOF1 (Treacher Collins-Franceschetti syndrome 1)
Van der Woude	IRF6 (Interferon regulatory factor 6)

22q11 deletion syndrome

The chromosome 22q11.2 deletion syndrome (Mendelian inheritance in man database number 188400) is a relatively common genetic disorder characterized by congenital cardiac defects, cleft palate, velopharyngeal insufficiency, distinct facial features, immunological problems, learning disabilities and psychological disorders. This syndrome is caused by a deletion of chromosomal material from the 22q11.2 region that leads to a wide but variable spectrum of clinical effects (24-27).

The commonly used method for the diagnosis of this deletion syndrome is FISH which is 95% sensitive and specific for the diagnosis of the 22q11.2 deletion (27). Management of cleft palate is not only the surgeons' job. A multidisciplinary cleft palate team is involved in this process. This team have to work together according to maximize the benefits to the patient.

Objectives

General Objective

To describe the clinical features, physical development, syndromic diagnosis, identification of 22q11 deletion syndrome and to develop cost effective screening method for detection of 22q11 deletion among cleft palate patients.

Specific Objectives

1. To describe the socio demographic background and clinical features of patients with cleft palate.
2. To describe the psychological status and physical development of patients with cleft palate.
3. To determine the prevalence of genetic syndromes associated with cleft palate.
4. To determine the prevalence of 22q11 deletion among patients with cleft palate.
5. To develop a cost effective molecular screening test to diagnose micro-deletion syndromes.

Materials and Methods

Subjects were identified among those who were currently under review in the Regional Cleft Centre & Maxillo-Facial Department, Teaching Hospital, Karapitiya, Galle. Subjects with cleft palate without cleft lip were selected for the study. General information including demographic data, educational information, occupation and family income, type of the cleft palate, associated clinical conditions, three generation pedigree analysis, antenatal history were collected. General clinical examination including developmental milestones, measurements of weight, height, head circumference, feeding in infants and speech in older children and adults were evaluated. Psychological status was evaluated by a designated psychologist. Ultrasound scans of the brain and abdomen, CT scans, echocardiograms, hearing and vision assessments and other investigations were obtained by review of reports.

Physical growth of the subjects below five years of age were analyzed by using a WHO Anthro version 3.2.2 (January 2011) designed and recommended by World Health Organization (WHO) for nutritional surveys. 3-5ml of venous blood from subjects were used for molecular genetic analysis.

Extraction of DNA was carried out according to a standard protocol and quantitative multiplex PCR was performed for each DNA sample to identify the 22q11.2 micro-deletion. PCR product quantitation was carried out in the log phase (within 22, 25 and 28 cycles) after electrophoresis using a gel documentation system (Bio Doc). All dosage estimations were carried out using 3 independent PCR reactions. A semi-quantitative method was used to measure the intensity of each band in the agarose gel. The intensity of each band was analysed (Table 4.3) using densitometric software “ImageJ 1.46r” (Wayne Rasband National Institute of Health, USA. Available at <http://imagej.nih.gov/ij>). A ratio of 1P:1N will indicate that there is no deletion while a ratio of 2P:1N will indicate a deletion.

7 DNA samples of 22q11.2 deletion and normal Subjects confirmed by FISH were used for the validation of semi-quantitative PCR. This study carried out in collaboration with Molecular Genetics and Biotechnology unit of University of Colombo and All India Institute of Medical Sciences, New Delhi, India.

Results

Gender distribution

There were three hundred and twenty three (n=323) subjects with cleft palate (without cleft lip), attended to the Regional Cleft Centre & Maxillo-Facial Department, Teaching Hospital Karapitiya, during the period from the 1st of January 2001 to 31st of December 2009. This included one hundred and eighty seven (n=187; 57.9%) females and one hundred and thirty six (n=136; 42.1%) males. All the 323 cases were invited to participate in the study. However, only one hundred and sixty two subjects (n=162; 50.14%) responded to the invitation to join the study. This group consisted of ninety five (n=95; 58.64%) females and sixty seven (n=67; 41.36%)

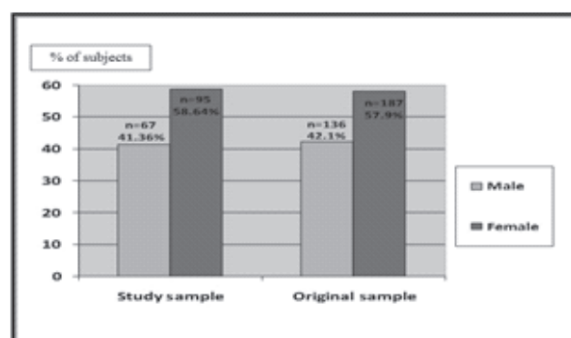


Figure 1 Gender Distribution

Table 2 Sex ratio

population	National figures (Southern province)	Study sample	p value
Sex ratio	0.96	0.71	0.0148

The male to female ratio (sex ratio) in the study sample was 0.71. According to the department of census and statistics in Sri Lanka the sex ratio in the southern province of Sri Lanka is 0.96 (Population and housing censuses in Sri Lanka, 2001).

There was a significant different ($p = 0.0148$) in sex ratio of the study sample (Table 2) and the national figure for the southern province.

The age of the study subjects ranged from two weeks to forty nine years. Twenty four (n=24; 14.81%) were less than one year of age and eighty three (n=83; 51.23%) subjects were less than five years of age. A total of one hundred and thirty three (n=133; 82.1%) subjects were below 12 years of age. The mean age of

the study population was 10.15 years while the median age was 9 years.

One hundred and forty six (n=146; 90.12%) subjects who participated for the study were resident in the Southern part of Sri Lanka. Eighty four (n=84; 51.85%) were from the Galle district, thirty nine (n=39; 24.07%) and twenty three (n=23; 14.20%) were from the Matara and Hambantota districts respectively. There were also eleven subjects from the Western province consisting of seven (n=7; 4.32%) from Kalutara district and five (n=5; 3.08%) from the Colombo district. Four subjects (n=4; 2.48%) were from the Ratnapura district in the Sabaragamuwa province.

There were one hundred and fifty one Sinhala (n=151; 93.2%), eight Moor (Muslim) (n=8; 4.9%) and three (n=3; 1.9%) Tamil subjects among 162 cases in the study group.

Table 3 Ethnic composition

Groups	Ethnic composition of the study sample**	Ethnic composition of the southern province of Sri Lanka*	p value
Sinhalese	93.9%	95.2%	0.09
Moor	4.7%	2.5%	0.007
Tamil	1.4%	1.8%	0.5
Other	0.0%	0.5%	

There was a significantly higher incidence of cleft palate among Moors (p = 0.007) compared with Sinhalese and Tamils in the southern province of Sri Lanka (Table 3).

There were eighty four (n=84; 51.85%) first rank births, fifty (n=50; 30.86%) second rank births, twenty (n=20; 12.35%) third rank births, six (n=6; 3.7%) fourth rank births and two (n=2; 1.23%) fifth rank births among study subjects.

Table 4 Birth Ranks

Birth rank	Percentage in the Sri Lankan population*	Percentage in study sample**	P value
1 st rank	43.19%	51.85%	0.0001
2 nd rank	34.79%	30.86%	0.07
3 rd rank	15.73%	12.35%	0.03
4 th rank	4.35%	3.7%	0.4
5 th rank	1.29%	1.23%	0.8

There was a statistically significantly higher incidence ($p = 0.0001$) of cleft palate among first rank births in the southern part of Sri Lanka (Table 4).

Type of clefts

There were one hundred and twenty five ($n=125$; 77.16%) subjects with cleft soft palate. Twenty five ($n=25$; 15.43%) subjects with cleft hard palate, seven ($n=7$; 4.32%) subjects with bifid uvula and five ($n=5$; 3.09%) subjects with submucous cleft palate.

Birth weight

The mean birth weight and prevalence of low birth weight of study subjects were compared with birth weight among the general population (The Sri Lanka demographic and health survey (SLDHS), 2006/07).

Table 5 Low birth weight

Population	Study sample*	Sri Lanka	p value
Percentage of low birth weight	38.8%	17.3%	$P < 0.0001$

There was a statistically significant ($p < 0.0001$) number of low birth weight among cleft palate subjects (Table 5).

Table: 6 Mean birth weight

Study	Present study	Mean Birth weight(28)	p value
Mean birth weight	2.73kg	2.85kg	0.003

There was a statistically significant difference ($p < 0.05$) in mean birth weight (Table 6) of the study sample and national figures (28).

Anthropometric measurements in less than five years old subjects

Anthropometric measurements of study subjects less than five years of age were analyzed. Weight, height, occipitofrontal circumference were recorded.

There were eighty three ($n=83$; 51.23%) study subjects less than five years of age. Physical growth of these study subjects was calculated according to the guidelines issued by World Health Organization (WHO) to assess the physical growth and development of the children less than five years of age using WHO anthro (version 3.2.2.) software.

Table 7 Distribution of the weight for age among cleft palate subjects

Parameter	<50 th percentile	%*	Expected %	>50 th percentile	%*	Expected %	P value
Total	69	73.13	50%	14	16.8	50%	< 0.0001

There was a statistically significant (one way chi-square test, $P < 0.0001$, chi-square 35.14) proportion of reduced weight for age among cleft palate subjects (Table 7).

Table 8 Distribution of height for age among cleft palate subjects

Parameter	<50 th percentile	%*	Expected %	>50 th percentile	%*	Expected %	P value
Total	55	66.26	50%	28	33.74	50%	0.0043

There was a statistically significant (one way chi-square test, $P < 0.01$, chi-square 8.14) proportion reduced height for age among cleft palate subjects (Table 8).

Table 9 Distribution of OFC for age among cleft palate subjects

Parameter	<50 th percentile	%*	Expected %	>50 th percentile	%*	Expected %	P value
Total	58	69.88	50%	25	30.12	50%	0.0004

There was a statistically significant (one way chi-square test, $P < 0.001$, chi-square 12.34) proportion of reduced OFC for age among cleft palate subjects (Table 9).

Table 10 Distribution of BMI for age among cleft palate subjects

Parameter	<50 th percentile	%*	Expected %	>50 th percentile	%*	Expected %	P value
Total	62	74.7	50%	21	25.3	50%	< 0.0001

There was a statistically significant (One way chi-square test, $P < 0.0001$, Chi-square 19.28) proportion of reduced BMI for age among cleft palate subjects (Table 10).

Types and distribution of other clinical conditions

Developmental delay ($n=23$; 14.2%) and dysmorphic features ($n=23$; 14.2%) were the commonest problems that were identified in the study population. Third commonest condition was cardiac abnormalities found in fifteen ($n=15$; 9.26%) subjects. Speech delay was found in twelve ($n=12$; 7.07%) subjects while there were five ($n=5$; 3.09%) each with hearing and central nervous system abnormalities. Four ($n=4$; 2.47%) had epilepsy. Genital, gastro intestinal and renal anomalies were found in two ($n=2$; 1.23%) subjects each. Visual abnormality was seen in one ($n=1$; 0.062%) subject.

Syndromes associated with cleft palate

According to the London Dysmorphology database there are more than 400 syndromes associated with cleft palate.

There were 14 subjects with identified genetic syndromes. Three subjects with Down syndrome, two with Edward syndrome, one with Patau syndrome and nine with rare genetic syndromes which includes a family with golden har syndrome, Apert syndrome, Femoral hypoplasia and unusual face syndrome, Fetal valproate syndrome, Multiple skeletal dysplasia syndrome, Fanconi anemia and Pier Robin syndrome. Identification of subjects with 22q11.2 deletion by semi-quantitative multiplex PCR

All ($n=162$) subjects with cleft palate were investigated to identify prevalence of the 22q11.2 deletion. In order to detect the deletion, dosage analysis using semi-quantitative multiplex PCR was performed using several sequence tag site (STS) markers within the deleted region. The cystic fibrosis transmembrane regulator gene (CFTR) was used as an internal control (sequence tag site markers in 7q31.2 region) in the same reaction. All PCR products were photographed and the intensity of each band was analysed using densitometric software “ImajgeJ 1.46r” (Wayne Rasband National Institute of Health, USA). Dosages of specific markers of patients (P) were compared to that of a non-deleted control (N) and a positive control with a deletion (C). A ratio of

1N: 1P indicated the absence of a deletion while a ratio of 1N:2P indicated a deletion. There were no subjects identified with 22q11.2 deletion in this study sample.

Cost effective molecular screening test to diagnose micro-deletion syndromes

7 DNA samples of confirmed 22q11 deletion and 7 DNA samples of confirmed without 22q11 deletion were tested by newly adapted semi-quantitative multiplex PCR method. All results were compatible with FISH test with 95% sensitivity and specificity.

Discussion

Cleft palate is one of the commonest malformations affecting the craniofacial region (4). It is associated with cleft lip and various other clinical manifestations. The London dysmorphology database includes more than four hundred syndromes associated with cleft palate (4). According to Tolarova and Cervenka, (1998) the birth prevalence of isolated cleft lip with or without cleft palate was 0.77 per 1000 live births and isolated cleft palate was 0.31 per 1000 live births. The non-Hispanic white population had the highest prevalence of isolated cleft palates compared to Asians and black populations (29). A study from the Central province in Sri Lanka using data from a tertiary care centre reported that the incidence of isolated cleft palate to be 0.19 per 1000 births (13).

In Sri Lanka, cleft palate patients are treated by oromaxillo-facial (OMF), plastic, paediatric and general surgeons. Cleft palate is one of the core features associated with 22q11.2 deletion syndrome and the 22q11.2 deletion syndrome is one of the commonest identifiable genetic causes of cleft palate (27). To the best of my knowledge there are no published data about the prevalence of 22q11.2 deletion in patients with cleft palate in Sri Lanka. The phenotype and genotype of this syndrome has not been defined in the Sri Lankan population.

Early detection of 22q11.2 deletion is important, as potential complications related to this syndrome can be treated prior to the cleft palate repair (27). Most of the patients with 22q11.2 deletion syndrome are diagnosed by clinical evaluation. The molecular investigations are essential to confirm the diagnosis of this syndrome. These investigations further facilitate the identification of atypical presentation of the syndrome. Data from Tobias et al., (1999) indicated

that about 41% of cases presented after the 2nd year of life and only 18% presented with the cardinal features such as conotruncal cardiac defects, velopharyngeal insufficiency, cleft palate or hypocalcaemia (27). Atypical presentation of the condition in 23% of subjects included isolated cardiac anomalies with family history of congenital cardiac disorders, psychosis and learning disabilities (27).

Early detection by a molecular diagnostic method is important to identify affected cases with 22q11.2 deletion syndrome in their early life because the pediatrician can screen for associated clinical manifestations of 22q11.2 deletion syndrome and manage them appropriately prior to the surgical intervention. It will also enable more accurate recurrence risk information to be given to the family (27).

In the current study there was a significantly higher prevalence of cleft palate among females. These results are keeping with the data of female predominance in other studies from Asia, Australia and parts of Europe (Table 11).

Table 11 Gender variation of cleft palate subjects in different populations

Study	Predominant gender of cleft palate group
Chuangsuwanich <i>et al.</i> , (1998) (30)	Female
Qiao-Juan <i>et al.</i> , (2006) (21)	Female
Linda <i>et al.</i> , (2002) (31)	Female
Womersley and Stone, (1987) (19)	Female
AL-Omari and AL -Omari, (2004) (32)	Female
Jagomagi <i>et al.</i> , (2010) (11)	Female
Current study	Female

The current study showed significantly low mean birthweight among cleft palate subjects (Table 12)

Table 12 Association between birth weight and cleft palate in different populations

Study	Association with birth weight
Ranalli <i>et al.</i> , (1975) (33)	No association
Boo and Arshad, (1990) (34)	No association
Fraser and Calnan, (1961) (35)	No association with male subjects. High risk of having low birth weight in female subjects
Wyszynski <i>et al.</i> , (2003) (36)	High risk of having low birth weight
Current study	High risk of having low birth weight

There were higher percentage of other associated clinical features among cleft palate subjects (Table 13).

Table 13 Percentages of other associated anomalies among subjects with cleft palate and cleft lip & palate in different populations.

Study/Population	Associated malformations (%)	
	Isolated cleft palate	Cleft lip and palate
Qiao-Juan <i>et al.</i> , (2006) (21)	2.18%	3.35%
Rajabian and Sherkat, (2000) (22)	18.34%	5.51%
Krumova, (2008) (37)	43.3%	Not recorded
Boo and Arshad, (1990) (34)	Not recorded	15.6%
Jagomagi <i>et al.</i> , (2012) (11)	Not recorded	30.3%
Womersley and Stone, (1987) (19)	65.89%	54%
FitzPatrick <i>et al.</i> , (1994) (38)	58.50%	34.53%
Jenson <i>et al.</i> , (1988) (39)	19.35%	Not recorded
Shprintzen <i>et al.</i> , (1985) (40)	Not recorded	63.4%
Current study	56.79%	Not recorded

There has been no formal and consistent methodology involved in identification of associated anomalies among cleft palate subjects in previously published research. Some studies had excluded syndrome diagnoses and chromosomal abnormalities (11,21,22) while other studies included associated malformations as a part of syndromic or other associated genetic condition (34,37-40). The present study population had other associated anomalies in more than half (56.79%) of cleft palate subjects which includes congenital heart defects, dysmorphic features, developmental delay, nervous system anomalies, hearing, visual anomalies and speech delay.

Data from current study indicated that there were higher incidence of growth retardation among cleft palate subjects and it is compatible with rest of the studies (Table 14)

Table 14 Physical growth of cleft palate subjects

Study	Growth of cleft palate subjects
Ranalli and Mazaheri, (1975)(41)	High incidence of growth retardation in the early months and subsequent catch up growth
Paradise <i>et al.</i> , (1974) (42)	High incidence of growth retardation in the early months and subsequent catch up growth
Avedian and Ruberg, (1980) (43)	High incidence of growth retardation in the early months and subsequent catch up growth
Pandya <i>et al.</i> , (2001) (44)	High incidence of growth retardation in the early months.
Lee <i>et al.</i> , (1997) (45)	High incidence of growth retardation in the nearly months and subsequent catch up growth
Current study	High incidence of growth retardation among cleft palate subjects below 5 years of age

In this present study there were no subjects found with 22q11.2 microdeletion by semi-quantitative multiplex PCR among cleft palate subjects in the Southern part of Sri Lanka. Based on this preliminary data, there is no specific indication to justify 22q11.2 deletion testing in the general cleft palate population (Table 15).

Table 15 Recommendation for screening of 22q11.2 deletion among all cleft palate subjects in different studies

Study	Recommendation for screening of 22q11.2 micro deletion among cleft palate subjects
Ruiter <i>et al.</i> , (2003) (46)	Screening for 22q11.2 deletion not recommended
Driscoll, (2001) (47)	Screening for 22q11.2 deletion not recommended
Stoll <i>et al.</i> , (2000) (48)	Screening for 22q11.2 deletion not recommended
Current study	Unable to give a definite recommendation at present

Conclusions and recommendations

According to the results of the current study following can be concluded

1. Female predominance in the cleft palate population in southern part of Sri Lanka.
2. Significantly higher incidence of cleft palate among Moor Ethnic population.
3. Significantly higher incidence of cleft palate among first rank births.
4. Significantly higher incidence of low birth weight among cleft palate subjects.
5. Cleft palate is associated with a number of other congenital anomalies.
6. Statistically significant higher proportions of cleft palate subjects with retarded physical growth.
7. Higher incidence of subjects with rare syndromes are associated with cleft palate
8. Semi-quantitative multiplex PCR method is reliable screening method for diagnosis of 22q11 deletion

At present, there is little evidence that routine molecular testing for the 22q11.2 microdeletion is needed for all patients with a cleft palate in the absence of other clinical features of 22q11 deletion syndrome.

Further investigations regarding other genetic causes of cleft palate may also be required to determine the most appropriate use of genetic testing in this resource limited developing country.

Multi-centered, collaborative research work is needed to generalize the results of this study to the rest of the Sri Lankan cleft palate population.

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Abstracts

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Impact of a 3-month combined exercise regimen on taste perception for sucrose in patients with type 2 diabetes mellitus

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Introduction

Physical exercise plays a key role in prevention and control of type 2 diabetes mellitus (T2DM). Although the importance of regular exercises on glycemic control is studied extensively, its impact on sweet taste perception (i.e. supra-threshold intensity ratings and preference) in T2DM is less reported.

Objectives

The aim of this study was to assess the impact of combined exercises (i.e. aerobic and resistance exercises) for 3 months on taste perception for sucrose in T2DM.

Methods

A sample of 127 T2DM patients aged 35-60 years was recruited and assigned randomly into two groups, an exercise group (n=64) and a control group (n=63). Demographic data were obtained using a pre-tested questionnaire. A graded exercise protocol was introduced to the exercise group i.e. aerobic exercises 4-5 days/week and resistance exercises 2-3 days/week for 3 months without changing diet and medications. Supra-threshold intensity ratings for sucrose were tested using 'General Labeled Magnitude Scale' and preference for sucrose was assessed by 'Monell 2-series, Forced Choice Method' in both groups at 0 and 3 months. Data were analyzed by Wilcoxon signed rank test. Significance was set at p=0.05 level.

Results

Exercise group showed increased supra-threshold intensity ratings for 4 out of 6 sucrose solutions with statistically significant results for 2 higher concentrations i.e. Difference in mean for 2.02M: $4.01/\pm 1.44$, $Z = -2.833$, $p = 0.005$ and for 0.64M: $6.83/\pm 0.55$, $Z = -2.650$, $p = 0.008$ when compared to their baseline values. Preference for sucrose was significantly reduced (Difference in mean = $0.03/\pm 0.01$, $Z = 2.569$, $p = 0.01$) after 3 months in the exercise group. The supra-threshold intensity ratings were not statistically significant in the control group while the preference for sucrose was significantly increased (Difference in mean = $0.1/\pm 1.03$, $Z = 2.483$, $p = 0.013$).

Conclusion

Taste sensitivity increases especially for higher sucrose concentrations and taste preference decreases in T2DM patients after 3 months of regular combined exercises.

Assessment of fluoride levels and hardness in commonly use drinking water sources among CKDu patients with tubulitis in Sri Lanka.

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Introduction

Symptomatic Chronic Kidney Disease of uncertain etiology (CKDu) with tubulitis is a recently described clinical entity. CKDu with tubulitis likely to be caused by recent exposure to the risk behaviours, nephrotoxin or both. Drinking water is often considered to be a major environmental source of nephrotoxic contaminants that cause CKDu. Therefore, assessing the water quality is a critical factor for evaluating reasons for CKDu patients with tubulitis.

Methods

46 biopsy confirmed CKDu patients with tubulitis from Girandurukotte, Wilgamuwa and Kandy renal clinics were recruited. Details of present drinking water sources were considered through a detailed interviewer administered questionnaire. Water samples were collected from all drinking water sources used by each patient. All the samples were collected according to the standard water collection methods. All together 60 water samples from different drinking water sources were analysed for fluoride and hardness. Fluoride levels were determined by ion chromatography (IC). The study showed that most of the patients (52%) use Protected Dug Well (PDW) for obtaining drinking water at home while 11% of patients used Reverse Osmosis (RO) water.

Results

According to the present study the median fluoride concentration in PDW was 0.53 mg/L while RO water showed the median fluoride level of 0.05 mg/L. 50% of patients consumed soft water while 38% of patients consumed moderately hard, 10% of patients and 2% of patients consumed hard and very hard water accordingly.

Conclusions

According to the study most of the CKDu patients with tubulitis consume dug well water and all the analysed chemical parameters such as Arsenic (As), Cadmium (Cd), lead (Pb), fluoride, magnesium and calcium were below the WHO recommended values. Therefore, according to the study findings inorganic contamination of drinking water sources seems unlikely to be the only cause of CKDu with tubulitis in this region, and further studies should be carried out to find the exact root cause for the disease.

Water drinking behaviour and contaminants of drinking water among CKDu patients in Wilgamuwa, Sri Lanka.

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Introduction

Chronic kidney disease of unknown aetiology (CKDu) is a progressive disease that causes irreversible renal failure and eventually the death. The aims of the study were to assess the drinking water quality, drinking behaviour and commonly use water sources among CKDu patients in Wilgamuwa, Sri Lanka.

Methods

A prospective cohort study was conducted for a study sample of 302 CKDu patients in Wilgamuwa, Data were collected through an interviewer administered questionnaire. Multiple water quality parameter values of 244 wells observed during the investigation. 95% of study participants believe that there is an association between CKDu and water source.

Results

All study participants believed that a nephrotoxic agent is carried by water. More than 50% of CKDu patients have changed the drinking water source after the diagnosis of CKDu. More than 50% of patients are using reverse osmosis (RO) water for drinking and cooking. This study results indicated high hardness values above threshold levels for human consumption according to WHO guidelines. The hardness values vary from 10 to 830 mg /L. The mean hardness value was 158.14 mg /L. Among the sample studied, fluoride concentrations of water consumed by 69 % of patients lies below 1 mg/l, which is considered as safe to consume. Al, Cd and As concentrations are almost comparable and below WHO recommendations.

Conclusions

This study results emphasise that trace elements concentrations in drinking water are less than WHO recommended values. Hardness of water is too high and majority of water sources are not safe for drinking purposes. Higher hardness of drinking water may cause many more health effects among consumers, therefore further studies should be carried out to identify any other health issues. Safe water should be supplied to this areas and awareness programmes on hard water should be conducted.

Evaluation of Midwives' knowledge on use of Edinburgh Postnatal Depression Scale (EPDS)- a study conducted in Galle district

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Introduction

Postpartum depression (PPD) affects 10–15% of women in childbearing age in western countries while higher prevalence rates are reported in South Asian countries.

PPD often goes unrecognized and remains untreated. This results in serious immediate and long-term adverse consequences for women, children, and their families.

Screening for PPD with the use of Edinburgh Postnatal Depression Scale (EPDS) is mandatory in the primary health care system. The effectiveness of screening is negatively affected by the primary health care worker's unfamiliarity and lack of proper training of the use of the tool.

Objective

To assess the knowledge of midwives on use of EPDS

Method

All midwives working in Galle district participated in this cross sectional descriptive study. Sample consisted of 254 participants. A self-administered questionnaire was used to obtain demographic data, duration of service and information on training they had on the use of EPDS. Five multiple choice questions each with four responses regarding use of EPDS was given and a score of 1 is given to each correct answer. Data were analyzed using statistical Package SPSS version 25.

Results

Majority (70.9%) of participants had >10 years of service. Written instruction and in-service training on use of EPDS were received by 81.1% and 53.9% of the participants respectively but 84.3% believed the training was inadequate. Regarding the use of EPDS, good knowledge was seen among 26% while majority (51.6%) showed satisfactory knowledge. 22.4% demonstrated poor knowledge. Satisfactory knowledge on use of EPDS showed significant associations with receiving written instruction ($p \leq 0.002$), undergoing training ($p \leq 0.01$) and having training conducted by a psychiatrist ($p \leq 0.03$).

Conclusion

There are key knowledge deficits with regard to use of EPDS in Midwives and regular training preferably by mental health professionals on administering the screening tool is recommended.

Dysglycaemia and acute anticholinesterase poisoning, an association?

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Introduction

Acute pesticide poisoning is a major health issue in developing countries. Among different types of pesticides used in agriculture, organophosphate (OP) and carbamate insecticides are commonly used in agrarian regions of Asia. In addition to cholinergic outcomes, hyperglycaemia has also been observed in few studies, due to ingestion of these poisons. The mechanism is not clear but stress and activation of hypothalamic pituitary adrenal axis, pancreatitis, oxidative damage and pleiotropic local effects have been suggested.

Objective

The aim of this study is to assess the random blood sugar (RBS) levels of subjects at the time of admission in relation to the severity of OP and carbamate self-poisoning.

Method

This prospective study was carried out with 60 (41 males) acute OP (n=24) and carbamate poisoned patients admitted to Teaching Hospital Anuradhapura. Patients admitted within 24 hours of poisoning and HbA1c level of <5.6 on admission were recruited. Severity of poisoning was measured by assessing Red Blood Cell (RBC) cholinesterase level measured prior to treatment with atropine. Decrease of RBC cholinesterase activity >50% (group 1), were considered as mild poisoning and decrease <50% (group 2), indicates severe poisoning. Data were analyzed using SPSS version 21.

Results & Conclusion

Mean age (SD) of total population was 34±13. Mean HbA1c was 5.2±0.4%. In-group 1 (n=33) mean RBS (SD) was 121±49 mg/dL and in-group 2 (n=27) mean RBS was 166±60 mg/dL and significantly higher (p=0.003). RBS levels inversely correlated with RBC cholinesterase activity (Pearson's r = - 0.341, p=0.008). The mean time between ingestion and measurement of RBS was (SD) 92±47 minutes. There was no significant difference of mean RBS levels on admission between OP (136±59mg/dL) and carbamate (145±59mg/dL). ingested patients (p=0.561). There is a correlation between acute anticholinesterases insecticide (OP and carbamate) poisoning and dysglycaemia.

“Colour cognition score”; a novel technique to assess colour cognition in children

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Introduction

Colour affect performance on cognitive tasks. Colours in the assessment of cognitive functions. However, there is no tool to assess the colour cognition (CC).

Methods

295 children with normal visual acuity and eight colour vision deficient (CVD) boys from the 12-15 age group were included. "Colour cognition score" (CCS) was determined in every participant. The participants were asked to name their choice of colour in response to a series of questions. Soon after the naming, they were asked to select colours from Manchester cognition score wheel corresponding to the same questions. Two adults with normal visual acuity and colour vision determined the congruousness of the naming and to the colours in two situations. If the naming and selections were the one mark was given. Zero marks were given for incongruousness. In this scale, colours 0 was the minimum and 7 was the highest marks. CCS of CVD boys were compared with age and sex-matched 32 boys randomly selected as the control group from the study group.

Results

There were 131 girls (mean age 12.9 (SD 1.1) years) and 164 boys (mean age 13.1 (SD 1.1)). Mean CCS was significantly lower in girls (4.2 (SD 2.1) than boys (5.0 (SD 2.1) ($p=0.001$). CCS was significantly low in 8 CVD boys (mean age 13.9 years (1.4 SD)) than 32 non-CVD boys (5.6 (SD1.6) vs. 6.7(0.6) $p=0.004$).

Conclusion

CCS assesses the colour cognition objectively. CC is significantly higher in male children. CC is impaired in CVD children compared to the age and sex-matched control group.

Identifying the factors affecting the efficacy of Povidone Iodine and comparison of one antiseptic agent Vs two antiseptic agents

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Introduction

Contamination of blood cultures with skin normal flora is an obstacle for the proper identification of pathogen. Seventy percent (70%) alcohol followed by 7.5% povidone iodine is used routinely in clinical practice for skin antisepsis when collecting blood for culture. Povidone iodine in high concentrations causes skin irritation.

Objectives

Comparison of one antiseptic agent vs two antiseptic agents in skin antisepsis and identifying the factors affecting efficacy of povidone iodine.

Methodology

A hospital based study was carried out enrolling 60 patients from medical wards using convenient sampling method. Swabs from both antecubital fossae of patients were collected before and 2 minutes after applying povidone iodine alone and 70% ethyl alcohol for 30 seconds followed by 7.5% Povidone iodine for 2 minutes and cultured in nutrient agar. Colony counts were taken after overnight incubation at 37°C. Mean reductions of colony counts in each occasion were calculated and compared using Independent t-test.

A laboratory based procedure was performed by adding 1ml of 0.5McFarland broth suspensions of *Staphylococcus aureus* and *Escherichia coli* into 9ml of different concentrations (2.5%, 5.0%, 7.5%, 10%) of povidone iodine and incubating at room temperature for different time periods (15 seconds, 30 seconds, 1, 2 and 4 minutes). 0.1ml of incubated solutions were subcultured on to nutrient agar and colony counts were compared after overnight incubation at 37°C.

Results

With one antiseptic, colony count reduction was 90.13% and with two antiseptic agents it was 93.42%. Mean colony count reduction was not significant between the two groups. ($P=0.534$, CI-13.79 to 7.22)

For *Staphylococcus aureus*, incubating with 2.5% PI for 2 minutes, 5.0% for 30 seconds, 7.5% for 01 minute and 10% for 15 seconds or more were more effective in killing. For *Escherichia coli*, incubating with 2.5%, 5.0% and 7.5% PI for 15 seconds or more and 10% PI for 2 minutes or more were more effective in killing.

Conclusion

For skin antisepsis, the use of 7.5% povidone iodine alone and 70% ethyl alcohol followed by 7.5% povidone iodine are equally effective. The lowest concentration and duration of incubation of povidone iodine to have an effective killing effect against *Staphylococcus aureus* and *Escherichia coli* is 5.0% PI for 30 seconds of time.

Antimicrobial effect of four medicinal plants used in Unani Medicine on selected bacterial pathogens

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Introduction

Infectious diseases have been a leading course of morbidity and mortality in the world. In western medicine antibiotics are used in the treatment of infectious diseases for decades. However, treatment with the antibiotics have become difficult, ineffective and complicated due to the increasing prevalence of multi – drug resistance in pathogenic microorganism as well as the undesirable side effects of antibiotics. Hence, there is a dire need to discover new antimicrobial agents with novel mechanism of action for new and re – emerging infectious diseases. In this respect, the plants used in Unani medicine could be explored as a potential source of new antimicrobials.

Objectives

The aim of this research is to identify the antimicrobial effect of four medicinal plants used in Unani medicine, i.e. *Cissus quadrangularis* (Heerassa), *Vernonia cinerea* (Monara kudumbi), *Mimosa pudica* (Nidikumba) and *Mikania cordata* (Vatupalu) against the standard cultures of *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853).

Methodology

The antimicrobial activity of the methanolic extracts of above plants were tested against standard cultures of *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* by the disc diffusion method according to CLSI guidelines. Ciprofloxacin (5 µg) and dichloromethane soaked filter paper discs were used respectively as the standard drug and negative control. The broth dilution method was employed to determine the minimum inhibitory concentration (MIC). Then minimum bactericidal concentration (MBC) was identified by plating the content of microplate wells in agar plates.

Results

Only the methanolic extract of *Mikania cordata* showed zone of inhibition against *E. coli* and *S. aureus* in disc diffusion method at the defined concentration of 2000 µg /mL. However, the broth dilution method resulted antibacterial effect in all four plant extracts with MIC of 250µg /mL against *S. aureus*, 62.5 µg /mL against *E. coli* and 500 µg /mL against *P. aeruginosa*.

Conclusion

The results of this study have shown that each tested plant extract exhibit antimicrobial activity against the tested bacteria.

Association between bone mineral density and body composition; a study involving pre and postmenopausal women

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Introduction

Local data on the association between bone mineral density (BMD) and body composition in relation to menopause are limited.

Objective

This cross-sectional study was performed to evaluate the association between hip BMD and body composition in two groups of women; premenopausal women (PrMW) and postmenopausal women (PMW).

Methods

Two groups of randomly selected healthy community-dwelling were studied separately as PrMW (n=184; aged 30-50 years) and PMW (n=166, aged 45-60 years) from Bope-Poddala MOH area. Hip BMD; femoral neck (FNBMD, g/cm²) and total-hip (THBMD, g/cm²), total-body-skeletal muscle-mass (TBSM, kg) and total-body-fat-mass (TBFM, kg) were measured with total-body DXA scans. Height (m), age (years) and period of menopause (POM, years) were recorded. Correlation, adjusted partial correlation and multiple regression analyses were performed.

Results

Mean(SD) ages of PrMW and PMW were 42.4(6.0) and 55.8(3.8) years respectively. In PrMW, FNBMD correlated positively with TBSM (r; 0.45) and TBFM (r; 0.42) (p<0.001). In PMW, FNBMD correlated positively with TBSM (r; 0.62) and TBFM (r; 0.58) (p<0.001). In PrMW, THBMD correlated positively with TBSM (r; 0.22, p=0.002) and TBFM (r; 0.27, p<0.001). In PMW, FNBMD correlated positively with TBSM (r; 0.58) and TBFM (r; 0.52) (p<0.001). Adjusting above correlations for possible confounders (age and height in both PrMW and PMW, and POM in PMW) did not change the results materially. TBSM and TBFM together explained 22% of variance of FNBMD in PrMW and 41% in PMW. Only the TBFM determined the THBMD in PrMW explaining 7% of variance while in PMW, it was determined by only TBSM that explained 34% of variance.

Conclusions

Both TBSM and TBFM have almost similar associations with the hip BMD independent of age and height in both PrMW and PMW, and POM in PMW.

Anticandidal activity of traditional Medicinal plants in Sri Lanka

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Introduction

Candida is an yeast that causes both superficial and deep fungal infections. Antifungals that are commonly used in the treatment are toxic and have become ineffective due to the development of resistance. In ayurvedic and traditional medicine, plant materials are used for the treatment of fungal infections.

Objectives

To determine the anticandidal activity of six selected medicinal plants i.e. Senna alata (Aththora), Curcuma longa (Turmeric), Sesbania grandiflora (Kathurumurunga), Phyllanthus emblica (Indian gooseberry), Psidium guajava (Guvava), and Cinnamomum verum (Cinnamon) against Candida albicans (ATCC 12420) and Candida glabrata (ATCC 90030).

Methods

Plants were selected from the Southern and North-Central provinces in Sri Lanka. Methanol extractions prepared from each plant were subjected to antifungal susceptibility testing (AFST) against both Candida species in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. Fluconazole and dichloromethane were used as the positive and negative controls respectively. The minimum fungicidal concentration (MFC) was determined for each plant extract by broth microdilution method.

Results

The initial concentration of 200 mg/mL of plant extracts showed no clear zone of inhibition, however a suppression of growth was observed around the discs of C. verum, P. emblica and P. guajava against C. glabrata and in C. verum against C. albicans. Hence MFC was determined with different concentrations of plant extracts (500- 31.25 mg/mL). An inhibition of growth of C. albicans was observed by C. verum, C. longa and P. guajava at the minimum concentration (31.25mg/mL) tested. An inhibition of the growth of C. glabrata was observed in S. grandiflora, C. verum, P. emblica and P. guajava at the minimum concentration (31.25mg/mL) tested.

Conclusion

The results of this study indicated the anticandidal activity in several selected plant extracts against both Candida albicans (ATCC12420) and Candida glabrata (ATCC 90030). Psidium guajava and Cinnamomum verum have good antifungal activity against both species thus they can be used as a drug or a topical ointment for candidal infections.

Comparison of the effectiveness of different substrates and culture media on Germ Tube (GT) production

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Introduction

The germ tube test (GTT) is a reliable method for presumptive identification of *Candida albicans* from other yeast species. Germ tube (GT) production is influenced by the type of substrate and the type of culture media.

Objectives

The objectives of this study were to compare the GT formation in five different substrates (Human serum- fresh; frozen; refrigerated and human plasma- fresh; frozen) and two different culture media (Blood agar-BA, Sabouraud dextrose agar-SDA).

Method

Standard *Candida albicans* control (ATCC 12420/M), already identified 14 isolates of *Candida albicans* and three non-*Candida albicans* species were selected for the study. GT production at different substrates and culture media was examined after 2 hours of incubation at 37°C. Average number of GT was calculated after examining ten high power fields (40*). Mean ranks, sensitivity and specificity of GT production were calculated at different substrates and culture media.

Results

There was a statistical significant difference on the effectiveness of GT production between the selected culture media ($p=0.04$). Sensitivity of SDA for GTT was 100% and BA was 86.67%. Both media showed 100% specificity. Sensitivity of five substrates used ranged 53.33% and 86.67% for GTT with 100% specificity. The highest mean rank of GT production was observed with fresh serum (4.68) while the lowest was recorded in refrigerated serum (1.61). Fresh serum, frozen serum and fresh plasma have similar sensitivity for GTT, it was about 86.67%. Further, they are more effective than fresh frozen plasma (53.33%) and refrigerated serum (60.00%). There was a statistical significant difference ($p=0.02$) between the other four substrates compared with fresh serum which is used for routine GTT procedure.

Conclusion

SDA worked as better culture media for GTT. The study findings confirmed that fresh serum, frozen serum and fresh plasma were better substrates for GTT.

Correlating platelet count to the onset of leaking phase among Dengue patients admitted to two selected medical wards in Teaching Hospital, Karapitiya (THK).

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Background

Almost all complications of dengue are due to the plasma leakage leading to reduce intravascular volume. During past few decades, Sri Lankan population has gone through several epidemics and currently experiencing annual dengue epidemics. The main objective of the study was to correlate absolute platelet count to the onset of leaking phase (LP) in Dengue patients.

Methods

Dengue was confirmed with either presence of positive NS1 or positive IgM antibodies. Serial ultrasonography was done on admission and twice a day until LP. Platelet counts (PC) were measured twice per day once count reached less than 150000/ μ L and blood samples were taken 30 minutes prior to each scan. Once the leaking detected latest PC was taken into the account.

Results

116 patients (age range 14-74, mean age 36.1 years (SD 13.9) were recruited. There were 47 (40.5%) females (age range 15-74, mean age 38.9 years (SD 14.9) and 69 males (14-69, 34.2 years (SD 13.1). LP was detected 3-7 days after onset of fever (4.9 days (SD 0.9). Mean Platelet count (MPC) at onset of LP was 30051 (SD 17023) / μ L (range 3000-77000). Age and the PC at the time of onset of LP was significantly negatively correlated in the group ($r = -0.323$ $p = 0.000$), females ($r = -0.369$ $p = 0.011$) and males ($r = -0.280$ $p = 0.020$). MPC at the time of onset of LP was significantly lower in patient more than 30 years than patients less than 30 years (27577.5 (SD 17361.4) vs. 33955.6 (SD 15888.8)).

Conclusion

The value of PC as a predictor of the progression of Dengue fever is dependent of the age of the patients. Considering the higher limit of the MPC at the onset of LP, leaking phase must be anticipated when the PC reach 80000 / μ L.

An audit on the effective management of the third stage of labour

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Introduction

Active management of the third stage of labour (AMTSL) is recommended in all patients undergoing vaginal delivery. AMTSL includes timely administration of IV syntocinon (AS), delayed clamping of the cord (DC), controlled cord traction and continuous uterine massage (CC). The main objective was to assess the current practice of the AMTSL against the standards.

Methodology

36 vaginal deliveries were observed in the labour room of tertiary care center. Steps followed according to the guidelines of management of the third stage of labour were noted.

Results

Mean age of the mothers were 28.2 years (SD4.2) (age range 19-36 years). There were 26 (72.2%) prime mothers. AMTSL has successfully completed in 24 (66.6%) deliveries. The completed components were 100% AS, 100% CC and 66.6% DC. Eight (2.5%) deliveries were associated with complications. There were 19.5% perineal tear and 2.8% Primary post-partum hemorrhages. The presence of complications was not different between deliveries with successful completion of AMTSL and non-completion of AMTSL ($p>0.05$).

Discussion

Delayed clamping of the cord is the least attended step in AMTSL. The factors affecting the non-completion of AMTSL needs further evaluation.

Association of sodium and potassium intake with blood pressure and anthropometric indices among healthy community dwellers

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Introduction

High sodium and low potassium intake in diet is known to increase the blood pressure (BP). Salt intake is higher than recommendations around the world.

Objective

Our aims were to estimate the dietary intake of sodium and potassium using urinary excretion and to find out their association with BP, anthropometric indices and demographic characteristics.

Methods

Demographic data were collected from 99 community living healthy individuals in Akmeemana MOH area. A 24-hour urine sample, BP and anthropometric measurements were obtained. Urinary concentration of sodium and potassium were estimated by direct Ion Selective Electrode method and dietary intake of sodium and potassium were calculated.

Results

Mean(SD) age of the participants (n=99) was 44(12) years. Median daily intake of sodium and potassium were 140.4 mmol (corresponding to 8.25 g NaCl) and 30.0 mmol (1.17 g) respectively. Sodium or potassium intake was not significantly different between males and females. Sodium intake was not significantly associated with age, educational status and occupation. Both sodium and potassium showed significant positive correlation with weight ($r=0.254$, $p=0.011$ and $r=0.285$, $p=0.004$) and waist circumference ($r=0.273$, $p=0.006$ and $r=0.298$, $p=0.003$). Only potassium was correlated with body mass index ($r=0.224$, $p=0.026$). Higher salt intake (≥ 5 g NaCl or ≥ 2 g Sodium daily) was reported among 67.7% of participants while only 3.03 % was reported to take adequate potassium (> 3510 mg/day). Sodium intake was not correlated with either systolic blood pressure (SBP) or diastolic blood pressure (DBP). Potassium intake was positively correlated with adjusted DBP ($r=0.208$, $p=0.039$) but not with SBP.

Conclusions

Dietary intake of sodium was higher and potassium was lower than recommended level and they were positively associated with certain anthropometric indices. However, sodium and potassium intake were not strongly associated with blood pressure and demographic variables of community living healthy individuals.

Health characteristics and living standards of prison inmates in Southern Sri Lanka

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Introduction

Identifying the health characteristics of prison inmates is important to improve the healthcare facilities provided.

Objectives

Our objectives were to describe sociodemographic data, health characteristics, psychological stress and living standards among prison inmates in Southern Sri Lanka.

Methods

This was a descriptive cross-sectional study conducted among prison inmates of a main prison in Southern Sri Lanka. A self-administered questionnaire, K10 psychological distress scale and a checklist formulated by International Committee of Red Cross (ICRC) for evaluating the living standards and medical records were used to collect data from the inmates.

Results

There were 845 inmates (828 males and 17 females) in the age range of 17-79 years and the median age was 33 years. Majority had received school education 809/838(96.5%) which includes 08/838 (0.9%) inmates with higher education. Most of them were skilled workers 401/798(50.3%). There were 560/840(66.7%) smokers, 525/840(62.5%) alcohol, 143/840(17.0%) cannabis and 107/840(12.7%) heroin users at the time of imprisonment. Psychiatric disorders, diabetes mellitus, hypertension, asthma, history of trauma and ischemic heart disease were reported in 148/845(17.5%), 72/845(8.5%), 60/845(7.1%), 46/845(5.44%), 33/845(3.9%) and 27/845(3.2%) inmates respectively. According to K10 scale 31.4% had experienced psychological stress. There were 05/845(0.6%) prisoners with smear positive tuberculosis. Score for living standards of the prison was 50.

Conclusion

Inmates were from young working group of the community. At the time of imprisonment psychoactive substance use was high. The proportion of inmates with non-communicable diseases was higher than communicable diseases. Living standards of the prison were in an acceptable level.

Knowledge, attitudes and practices about cervical cancer among school teachers in Elpitiya education zone

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Introduction

Cervical cancer remains the second most common gynaecological malignancy in Sri Lanka. Cervical cancer screening (Pap test) and school-based HPV vaccination programme are cervical cancer preventive programmes available in the country. Teachers can play a major role in creating awareness among students and their parents to improve coverage of these preventive programmes.

Objectives

The study objectives were to describe the knowledge, attitudes and practices on cervical cancer and its prevention and their associated factors among female secondary school teachers in Elpitiya education zone.

Methods

A descriptive cross-sectional study was conducted among 630 female school teachers in Elpitiya education zone. A pre-tested, structured, self-administered questionnaire was used to collect data on demography, knowledge (on risk factors, symptoms and screening services), attitudes and practices on cervical cancer and its prevention. For each question scoring was done as 1 for a correct answer and 0 for an incorrect answer. Overall knowledge score was transformed into a 0-100 scale and three categories were defined as 'poor':<35; 'average':36-70 and 'good'>71 using visual binning. Comparisons were made using Chi-squared tests and p value <0.05 was considered significant. Ethical clearance was obtained from the Ethics Review Committee of the Faculty of Medical Sciences, University of Sri Jayewardenepura.

Results

'Poor' knowledge levels were observed on symptoms (N=276, 49.4%) and risk factors (N=352, 62.6%) of cervical cancer. Longer experience in teaching (p=0.014) and higher family income (p=0.009) showed statistically significant associations with higher knowledge. Most (N=490, 88.6%) were willing to get their daughters vaccinated in school-based HPV vaccination programme. Participation in Pap screening was 48% and was associated with higher knowledge (p=0.001). The main reason for non-participation was being busy in the school (32%) followed by perceived lack of need (13%) and the procedure being perceived as embarrassing (11%) or painful (11%).

Conclusions

Knowledge, attitudes and practices on cervical cancer and its prevention among female school teachers need to be improved.

Prevalence and associated factors of musculoskeletal problems among tea estate workers in Homadola estate, Galle

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Introduction: Musculoskeletal disorders (MSD) are generally believed to be high among estate workers. Approximately 4.4% of the Sri Lankan population lives in tea estates. There are inadequate research studies regarding occupational influence on MSD. The objective of this study was to assess the prevalence and associated factors of musculoskeletal problems among tea estate workers in a selected tea estate in Galle district.

Methods: A cross-sectional study was conducted among tea estate workers at Homadola estate Udugama, Galle and the sample size was 210 including male (11.9%) and female (88.1%) workers. An interviewer-administered questionnaire was used to collect data. Involvement of more than two joints was considered as positive for MSD in this study.

Results: The prevalence of MSD was 67.1%. Most commonly affected areas were back (93.3%), knee joint (83.7%) and shoulder joint (71.9%). Being a female (Chi square = 6.890, $p = 0.009$) and being a tea plucker (Chi square = 9.232, $p = 0.01$) were significantly associated with increased risk for MSD. Disturbances in day to day activities were reported by 42.1%, whereas 51.9% experienced difficulty during working hours and 51% had to obtain leave from work. Western medicine was the most common (89.5%) health seeking behaviour for MSD among tea estate workers.

Conclusions and Recommendation: Prevalence of MSD is high in tea estate workers of Homadola estate and it affects their working capacity. The results indicate the need to identify possible underlying factors such as improper working behaviour and to implement appropriate measures that would prevent MSD among tea estate workers.

Key words: Musculoskeletal disorders, tea estate workers, prevalence, Galle

Completeness of laboratory request form filling and the perceptions regarding request form filling among medical officers in Teaching Hospital, Karapitiya

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Background: Request forms, filled by Medical Officers, are the first contact between patient and laboratory. Their completion has a huge impact on test results. Several factors affect the incompleteness of request forms. This study evaluated the completeness of laboratory request forms received by the laboratories and perceptions regarding request form filling among Medical Officers (MO) of Teaching Hospital, Karapitiya (THK).

Methods: A cross-sectional study was conducted in two components. To assess the completeness of request forms, 384 request forms received by Chemical pathology, Haematology, Histopathology and Microbiology laboratories of THK were analyzed retrospectively. For assessment of perceptions of MOs regarding completion of request forms, 284 MOs were recruited using stratified random sampling. Data were collected using a self-administrated questionnaire and analyzed using SPSS software.

Results: Overall completeness of request forms was zero and only 30.2% had a satisfactory level of completion according to ISO 15189 standards. The least completed areas were clinical history (50%), clinician's name (27.6%) and time of sample collection (5.6%). Approximately 4% of the requests were made using incorrect request form. Completeness was not associated with urgency of investigation ($p>0.05$). Perception of MOs regarding request form filling was satisfactory, however, only 43% of MOs completely filled the request forms, the main reason being lack of time (83.9%).

Conclusions: Completeness of request forms received by the laboratories of THK is not satisfactory. Perception of MOs regarding request form filling was satisfactory, although there was a discrepancy with their form filling behavior, mainly due to lack of time. Measures should be taken to minimize the difficulties faced by the MOs during request form filling.

Keywords: Laboratory, Request forms, Medical Officers, Completeness, Perceptions

Knowledge, attitudes and practices on over-the-counter skin whitening creams among female Advanced Level school students in Galle Education Zone

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Introduction: Various skin lightening products are available in Sri Lankan market that are freely available over-the-counter and are cheap. In the current society, a huge trend has been developed among younger population in using skin whitening products regardless of their content and adverse effects. This study aimed to identify the knowledge, attitudes and practices on over-the-counter skin whitening creams among female Advanced Level (A/L) students in Galle Education Zone.

Methods: A cross-sectional study was conducted among 400 female A/L students in Galle Education Zone, selected by using non-probability multistage sampling method. Data were collected using a self-administered questionnaire and analyzed using SPSS statistical software.

Result: Of the participants, 172 (43%) were using skin whitening products. Most popular whitening cream was a local brand Chandanalepa®. Most influential factors for purchasing whitening creams were ingredients, friends and siblings, brand and media. Major access points to the products were pharmacies and supermarkets. A considerable number developed adverse effects following usage, the commonest being acne (82.5 %) and skin irritation (22.2%). Of the respondents, 69.3% were aware on ingredients in skin whitening products. Students who were using these products had a positive attitude on whiter skin ($p < 0.05$).

Conclusions and recommendations: A considerable proportion of students use whitening cream without proper guidance and they had a positive attitude towards a lighter skin. Despite the knowledge on ingredients, students continued to use skin whitening products. Considerable number of students had experienced side effects. Tightening and improving rules and regulations regarding marketing of over-the-counter whitening products should be done. It is better to add information on cosmetics and related health effects to school health education.

Key words: Knowledge, attitudes, practices, skin whitening creams, adverse effects

Prevalence and associated factors of respiratory diseases among traffic police officers in Galle district

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Introduction: Traffic police officers are continuously exposed to toxic air pollutants because of the nature of their work and are therefore more prone to get respiratory diseases. The study aimed to assess the prevalence of the respiratory diseases and associated factors among traffic police officers in Galle district.

Methods: A cross-sectional survey was conducted among 86 traffic police officers in Galle district, selected using convenience sampling. Data were collected using a self-administrated questionnaire and peak flow meter readings and analyzed using IBM SPSS statistical software.

Results: The overall prevalence of respiratory diseases among traffic police officers was 39.7%, whereas prevalence of chronic cough, chronic phlegm, wheeze and dyspnea was found to be 32%, 37.2%, 14% and 27.9% respectively. Gender, past history of respiratory diseases, use of personnel protective equipment and working hours near roadside per day were significantly associated with the presence of respiratory diseases. However, no significant association was observed between respiratory diseases and the age, place of residence, housing characteristics, exposure to smoking, family history of respiratory diseases, working location, working years, number of years in current post and shifts of duty. There was no significant impact of the respiratory diseases on their occupation and life style.

Conclusions and recommendations: Traffic police officers have a considerably high prevalence of respiratory diseases. Regular monitoring of traffic police officers, encouraging them to use personal protective equipment during working hours in road and improving their knowledge on risk factors would help in early identification and reduction of risk of getting respiratory diseases among traffic police officers.

Key words: respiratory diseases, traffic police officers, Sri Lanka

Knowledge and attitudes regarding Basic Life Support among primary health care workers in Galle district

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Introduction: Basic Life Support (BLS) is considered as the initial level of medical care, used in saving life of a person. Cardio Pulmonary Resuscitation (CPR) has been considered as the role play in BLS. As primary health care workers interact with the community often, the basic knowledge regarding CPR should be virtually known by all primary health care workers. Objective of this study was to assess the knowledge and attitudes on BLS among primary health care workers in Galle district, focussing mainly on CPR.

Methods: This cross-sectional study was conducted among 250 primary health care workers in 10 Medical Officer of Health offices in Galle district, excluding the supportive staff. Non-probability, consecutive sampling was used and a pre-tested, self-administered questionnaire was used for data collection. The level of knowledge was determined by assigning scores for correct responses. The data were analyzed using IBM SPSS software.

Results: Of all participants, 2.2% (n=5) had adequate knowledge on CPR, whereas 94.7% were aware, 51.3% had observed and 20.8% had performed CPR during their day-to-day life. The attitudes towards CPR was excellent in majority (99.1%, n=224). Approximately 89% believed that their knowledge on CPR is not adequate which was also evident with their scores. The proportion who had claimed to have adequate knowledge (8.8%, n=20), however did not achieve satisfactory scores in their assessment of knowledge.

Conclusions and Recommendations: Primary health care workers in Galle district had good attitudes towards CPR; however, their knowledge on CPR is not satisfactory. Regular training should be incorporated to already aligned training workshops in local setting.

Key words: Basic Life Support, Cardio Pulmonary Resuscitation, knowledge, attitudes, primary health care workers

Awareness and practices related to use and storage of pharmaceuticals among patients with chronic diseases attending Teaching Hospital Karapitiya

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Introduction: With the increasing prevalence of chronic diseases, domestic drug utilization has drastically increased. As a result, irrational drug usage and improper and unsafe drug storage has been increasing globally in both developed and developing countries, giving rise to a high rate of unwanted effects of pharmaceuticals. The objective of this study was to assess the awareness and practices related to use and storage of pharmaceuticals among patients attending Teaching Hospital, Karapitiya.

Methods: A clinic-based, cross-sectional study was conducted among 350 patients suffering from selected chronic diseases attending general medical clinics of Teaching Hospital, Karapitiya. Data were collected through an interviewer-administered questionnaire and analyzed using SPSS statistical software.

Results: Among the participants only 38 (10.8%) had a satisfactory awareness regarding all five essential aspects of personal medication. Older patients had a significantly less awareness than younger patients (8.0% Vs. 24.6%; $p < 0.01$). Participants with a higher education level (above GCE O/L) had significantly higher awareness compared to those studied below O/L (16.3% Vs. 4.9%; $p < 0.01$). A considerable proportion of participants (>70%) were aware regarding proper storage of medicine. Out of all the patients using emergency medications, 96% could easily access their emergency drugs. Practice of disposal of their drugs after expiry was considerably low (35.7%) among the participants.

Conclusions and recommendations: The patient population attending hospitals clinics appear to be poorly informed regarding safe and proper storage and usage of their medication. A systematically organized patient education system can further improve the awareness and practices of use and storage of pharmaceuticals.

Key words: Rational drug use, safe storage, pharmaceuticals, medications, awareness, practices

Quality of life and associated factors in patients with Rheumatoid Arthritis treated at Teaching Hospital Karapitiya

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Introduction: Rheumatoid arthritis (RA) is a chronic autoimmune disease which affects the quality of life (QOL) of an individual causing severe disability. QOL is an important measure especially in diseases with a chronic course and is an indicator of a patient's perception rather than an assessment done by care providers. World Health Organization Quality of Life- BREF (WHOQOL-BREF) is a questionnaire that assess QOL in four domains; physical, psychological, social and environmental health. This study aimed to assess the QOL and its associated factors in rheumatoid arthritis patients attending Teaching Hospital, Karapitiya.

Methods: A cross-sectional study was carried out in a sample of 210 patients (males=17.1%, females=82.9%) using non-probability quota sampling. Self-administered and interviewer based WHOQOL-BREF questionnaire was used as a study instrument. Data analysis was done using SPSS statistical software to obtain the scores for different domains of QOL. Chi square test, Fisher's exact test and independent sample t test were used to assess the association between QOL and selected factors.

Results: The mean age was 57.8 years (SD=10.6 years). Out of 210, 40.5% belonged to social class five. QOL was poor among 86.7%. Interpersonal relationships and the level of independence were not significantly affected by RA. There was no statically significant association between age and QOL ($t=0.601$, $p=0.854$), gender and QOL (Fisher's exact test=0.793), social class and QOL (Chi square=1.133, $p=0.568$), gender and interpersonal relationships (Chi square=3.337, $p=0.068$) and gender and physical activity (Chi square=0.969, $p=0.325$) according to our study.

Conclusions and recommendations: RA is associated with poor QOL, however the basic socio-demographic factors assessed in this study had no direct effect on QOL.

Key words: Quality of life, Rheumatoid arthritis, associated factors

Prevalence and Associated Factors of Chronic Widespread Pain among Nurses in Teaching Hospital, Karapitiya

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Introduction: American College of Rheumatology defines Chronic Widespread Pain (CWP) as pain in the left and right side of the body as well as above and below the waist and pain in the axial skeleton. Our study aimed to assess prevalence and associated factors of chronic widespread pain among nurses in Teaching Hospital, Karapitiya. Above mentioned criterion along with persistent pain at least for 3 months was taken as the criteria in our study to detect CWP.

Methods: A cross-sectional study was conducted among 400 nurses selected conveniently from all the wards, specialized units and clinics at Teaching Hospital Karapitiya. Data were collected using a self-administered questionnaire and analyzed using the IBM SPSS statistical software.

Results: According to our study 12.2% of the sample was suffering from CWP with a female: male ratio of 1.3 to 1.0. Among individuals with CWP, 89.6% complaint of knee pain, while 81.3% suffered from back pain. Approximately 20% of the participants had both CWP and non-communicable diseases (NCDs). Associated factors considered were age, gender, marital status, service period in years, daily working hours, overtime hours per month, all NCDs and working units. There was a significant association between CWP and NCDs ($p < 0.05$), whereas other associated factors did not show a relationship with CWP.

Conclusions and recommendations: The prevalence of CWP was low among nurses in this sample, with a slightly higher prevalence in females. Knee pain and back pain were the commonest types of pain. There is a relationship between CWP and NCD. Further studies are recommended to explore the association of CWP and NCD and causes of knee and back pain.

Key words: Chronic widespread pain, prevalence, nurses

Satisfaction regarding quality and availability of food services among medical undergraduates of University of Ruhuna

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Introduction: University life is where students make their new food adaptations which can have a great impact on their future food habits and health. Food and nutritional problems and unsatisfactory quality of food are particularly prevalent among the university students, which may affect their health and well-being. The aim of our study was to assess the level of satisfaction regarding quality and availability of food services among medical undergraduates of University of Ruhuna.

Methods: A cross-sectional study was conducted among 384 undergraduates in Faculty of Medicine, University of Ruhuna, selected to represent all years of study. Data were collected using a self-administered questionnaire and analysed using SPSS software.

Results: The commonest source of food was Faculty canteen (73.4%) and 25% obtained meals from local food sellers and restaurants. Only 15.6% were satisfied about the overall nature of food. Participants were mostly unsatisfied about the nutritional value (13%) and hygienic aspects (16.7%) of food. In comparison, the odour (33.3%), appearance and service hours (both 32.3%) of food were the most satisfactory aspects. Of the sample, 47.5% had experienced some food borne illness after consuming food.

Conclusions and recommendations: Level of satisfaction regarding the food services is very low among medical undergraduates and is mainly affected by nutritional and hygienic aspects. There was a considerably high prevalence of food borne illnesses among the undergraduates. These finding suggest the need for regular monitoring and evaluation of food services for improvement of quality and prevention of food borne illnesses.

Key words: Food services, quality, availability, satisfaction, undergraduates

Assessment of knowledge on basic First-aid of primary school teachers in Galle Municipality

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Background: Injuries are common and can occur at any point of time in a day. Unintended injuries in primary school children need immediate lifesaving care which is known as first-aid. This study aimed to assess the knowledge on basic first-aid of primary school teachers in Galle Municipality.

Methods: A cross-sectional study was conducted among 388 primary school teachers in Galle municipality. Data was collected using a pre-tested, self-administered questionnaire and analyzed using SPSS (Statistical Package for Social Sciences). Association between variables was determined using chi-square test.

Results: All 388 teachers participated in the study with a response rate of 100%. Among them 71.4% were found to be knowledgeable on first-aid measures and only 13.7% had previous training. Having a previous training had no association with current first-aid knowledge. The majority (98.5%) believed that having basic first-aid knowledge is necessary for them and 91.8% had a positive attitude towards giving a basic first-aid training/workshop to them.

Conclusions and recommendations: Majority of the teachers were knowledgeable and have positive attitudes towards first-aid. Previous first aid training had no association with current first aid knowledge. Media is an effective way to reach the general public. Therefore respective authorities should aim to fortify the basic first-aid knowledge through media. Assessment of first-aid skills is another important area for future research.

Key words: Basic first-aid, knowledge, training, primary school teachers, Galle

Prevalence of overweight and obesity among medical undergraduates of University of Ruhuna and its association with stress

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Introduction: Medical undergraduates experience a substantial amount of academic stress. In contrast with other countries, we lack published data to assess whether there is an association of stress with overweight and obesity among medical students in Sri Lanka. This study assessed the prevalence of overweight/obesity and its association with stress among medical undergraduates of University of Ruhuna, Sri Lanka.

Methods: A cross-sectional study was conducted among 258 medical students (62.4% female) in Faculty of Medicine, University of Ruhuna, selected using stratified random sampling. A self-administered questionnaire and a data record sheet were used as study instruments. Anthropometric measurements were taken according to standard protocols. Data were analyzed using SPSS statistical software.

Results: Approximately 27.8% male and 6.8% female students were overweight/obesity {Body Mass Index (BMI) of $\geq 25 \text{ kg/m}^2$ } affecting an overall proportion of 14.7%. Central obesity was seen in 29.9% of males and 37.9% of females. Prevalence of central obesity was higher in para-clinical group (45.7%) than preclinical group (24.4%). Of the students with overweight/obesity, 39.5% had unhealthy dietary habits and approximately 38% reported very low physical activity levels. Nearly 94% of all medical students and 89.5% of overweight/obese students had a high or moderate stress level. There was a non-significant negative correlation between stress level and BMI ($r = -0.122$, $p > 0.05$).

Conclusions and recommendations: There is a high prevalence of central obesity among medical students of University of Ruhuna with an alarmingly low level of physical activity. The prevalence of stress was significantly high in all BMI groups. Measures should be taken support weight management and reduce stress levels while improving dietary habits and physical activities of students.

Key words: Overweight, obesity, stress, medical students, dietary habits, physical activity

Awareness of risk factors and early detection of breast cancer in women of reproductive age attending Teaching Hospital Karapitiya

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Introduction: Breast cancer is the most common cancer in urban women. Shyness and not knowing self-breast examination, ignorance about symptoms of breast cancers and social stigma of cancer along with financial constraints are the main factors responsible for delayed presentation. This study aimed to assess awareness of risk factors, symptoms of breast cancer and knowledge & practices on breast self-examination in women of reproductive age attending Teaching Hospital, Karapitiya.

Methods: We conducted a cross-sectional study recruiting 362 consenting women who attended the Outpatient department of Teaching Hospital Karapitiya. Data was collected using a pre-tested, self-administered questionnaire and analyzed using descriptive statistics as means, proportions and inferential statistics such as Chi square test, independent t-test and correlation using SPSS statistical software. The level of awareness was assessed using a scoring system based on the answers provided for the questionnaire.

Results: The mean age \pm SD of the respondent was 34.6 ± 9.2 years and 71.9% had a poor knowledge on risk factors of breast cancer. Seventy two percent of the women were aware of self-breast examination; however, only 53.7% practiced it. Of those who practiced self-examination only 12% used the correct method.

Conclusion: The current level of awareness regarding risk factors and early detection of breast cancer and the practice of self examination of breast is not satisfactory among women in reproductive age. Steps should be taken to improve the level of breast cancer awareness and screening among target females in the community.

Key words: Breast cancer, breast self-examination, awareness, risk factors, reproductive age

Prevalence of allergic rhinitis and rhinoconjunctivitis among 5-12 years children in Bope-Poddala Medical Officer of Health area

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Introduction: Allergic rhinitis and rhinoconjunctivitis is a common chronic disorder in children which is frequently under diagnosed, misdiagnosed, mistreated or ignored. Despite being a global health problem, especially in developing countries, it has not been investigated adequately using epidemiologic approaches. The objective of this study was to identify the prevalence of allergic rhinitis and rhinoconjunctivitis, its triggering factors and impact on school attendance and vision among 5-12 year-old children in Bope-Poddala Medical Office of Health (MOH) area.

Methods: A cross-sectional study was conducted on a random sample of 260 children aged 5-12 years in Bope-Poddala MOH area. An interviewer-administered questionnaire was used to collect data on study variables. Data were analyzed using Statistical Package for Social Sciences (SPSS).

Results: Prevalence of allergic rhinitis and rhinoconjunctivitis among 5-12 year-old children in Bope-Poddala MOH area was 21.5% and there was no gender difference. Triggering factors in most of them were dust (20%), cold air (17%) and ice cream (17%). School attendance was affected in 60.7% of the children with this condition. There was no association between impaired vision and allergic rhinitis and rhinoconjunctivitis in this sample, however, a moderate association was observed with bronchial asthma ($p < 0.05$).

Conclusions and recommendations: Allergic rhinitis and rhinoconjunctivitis is a prevalent health issue among 5-12 year-old children in Bope Poddala MOH area and are mostly associated with environmental factors. Health education to improve awareness of this disease and limitation of exposure to triggering factors would be helpful to reduce the prevalence of this disease.

Key words: Allergic rhinitis, rhinoconjunctivitis, prevalence, triggering factors, impact, children



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