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Editorial

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Need for a Journal of Physiology in Sri Lanka

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There has been a significant growth in the field of physiology in Sri Lanka over the past few decades. This progress could be attributed to the establishment of the Physiological Society of Sri Lanka in 1987, which provided a structured platform for the advancement of physiological sciences.

Here are some key points highlighting the need for a dedicated Journal of Physiology in Sri Lanka:

1. Expanding Educational Landscape: The number of medical, dental, and related faculties offering physiology courses has increased significantly. This indicates a growing interest and investment in physiological education and research.

2. Diverse Degree Programs: The introduction of postgraduate programs, including the Postgraduate Diploma in Physiology and MPhil/PhD programs, demonstrates a maturing academic environment. This not only attracts more students to the field but also contributes to the overall research capacity.

3. Specialization and Research Diversification: The emergence of diverse fields within physiology, from basic to clinical physiology, indicates a deeper level of specialization and expertise. This diversification necessitates a platform for sharing and disseminating this specialized knowledge.

4. Research Capacity: The development of research capacity in physiology is a crucial factor. This suggests that there is a wealth of research being conducted, which needs an appropriate outlet for publication.

5. Fulfilling a Gap in the Current Journal Landscape: While there are journals in Sri Lanka covering medical and allied sciences, the absence of a dedicated journal for physiology is a clear gap. A specialized journal can provide a focused platform for physiologists to publish their work.

6. Fostering a Scientific Community: A dedicated journal can help foster a sense of community among physiologists in Sri Lanka. It can facilitate networking, knowledge exchange, and collaboration among researchers and educators in the field.

In summary, the growth and diversification of physiology in Sri Lanka, along with the expanding educational and research landscape, underscore the need for a dedicated Journal of Physiology. Such a journal would serve as a vital platform for publishing and disseminating research findings, fostering collaboration, and further advancing the field of physiology in Sri Lanka.

It is our fervent hope that the Sri Lanka Journal of Physiology would fill this vacuum and provide necessary platform for the advancement of the field of Physiological Sciences in Sri Lanka.

Sri Lanka Journal of Physiology **SLJPHYSIOL**

Original Research

Citation: Yasawardene PC, Silva AS De & Atapattu PM, 2022. Quality of sleep of medical students undergoing online medical education at the time of the COVID-19 pandemic in a selected medical faculty in Sri Lanka. Sri Lanka Journal of Physiology, pp 3-9

Quality of sleep of medical students undergoing online medical education at the time of the COVID-19 pandemic in a selected medical faculty in Sri Lanka

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Abstract

Background: Sleep is a vital yet often disregarded physiological process required for good physical and mental health.
Objectives: This study aimed to assess the sleep quality and associated factors among medical students undergoing online medical education in a selected Sri Lankan medical faculty.
Methods: A cross-sectional analytical study was conducted among all current medical students of the Faculty of Medicine, University of Colombo, using a self-administered online questionnaire incorporating Pittsburgh sleep quality index (PSQI).
Results: 331 valid responses were received. 52.3% were females. 31.4% were first year students. Majority (55.9%) spent ≥6 hours/day on online education. The mean (±SD) PSQI score was 6.21(±2.84). Sleep quality was poor (PSQI score≥5) in the majority (71.3%), worst among first year students (86.5%). Mean (±SD) sleep duration was 6.4 (±1.4) hours and lowest (5.7(±1.3) hours) among first year students. 59.5% of all participants slept <7 hours. 7.3% used sleep medication at least once in past month. 39.0% reported having trouble staying awake while driving/eating/during social activity at least once in past month. Significantly higher poor sleep quality was observed in first year students vs. third/fourth year students ($p<0.05$) and significantly increased with daily time spent on online education ($p<0.05$). Poor sleep quality was not associated with gender/time spent on non-academic computer activities.
Conclusions: Poor sleep quality is highly prevalent among medical undergraduates, especially first year students, and is associated with the time spent on online education. This may have adverse physical and mental health consequences and needs prompt investigation and action.

Keywords: Sleep quality, Online, Medical Education

INTRODUCTION

Sleep is a vital physiological phenomenon required for good physical and mental health. Inadequate sleep can give rise to both physical and mental disorders [1]. Medical students are known to have poorer sleep quality compared to other university students, which may be due to increased intensity of study and challenging clinical responsibilities [2]

The COVID-19 pandemic has caused major changes in education, especially in developing countries. Medical undergraduates in Sri Lanka had traditional classroom-based teaching shifted completely to online platforms, allowing students to be independent learners [3], but also subjecting them to the negative effects of increased screen-time.

Increased screen time, especially in the hours before sleep, is associated with poor total sleep duration and sleep quality, delayed bedtime, and increased daytime sleepiness [4, 5]. The COVID-19 pandemic has disrupted sleep patterns and sleep quality in humans [6]. The impact of unprecedented increase in the screen time due to online learning on the sleep quality of medical students is an important area yet to be fully explored.

This study was carried out with the aim of evaluating the sleep quality and associated factors among medical students undergoing online medical education amidst the COVID-19 pandemic at one medical faculty in Sri Lanka.

MATERIALS AND METHODS

This was a cross-sectional, analytical study at the Faculty of Medicine, University of Colombo, Sri Lanka. Permission was obtained from the Dean, Faculty of Medicine and ethical approval was granted by the Ethics Review Committee, Faculty of Medicine, Colombo (EC-21-106)

Medical students across all years comprising six batches were included. Sample size was calculated for the population of 1200 current students, requiring a minimum sample size of 291. Data was collected in October 2021, via an online self-administered questionnaire comprising components on socio-demographic and economic data and Pittsburgh Sleep Quality Index (PSQI) questionnaire [7], validated for Sri Lanka [8]. The PSQI assessment was limited to the past month from the date of data collection.

STATISTICAL ANALYSIS

Data analysis utilized SPSS statistical software package IBM SPSS Statistics for Windows, Version 25.0 (Armonk, NY: IBM Corp.). PSQI score $\geq$5 was considered as poor sleep quality. Chi-squared test with 95% significance level was used for associations with post-hoc analysis using z-tests of independent proportions for column percentages with Bonferroni correction.

RESULTS

Results of the 331 participants, the majority were females (52.3%). The highest and lower lowest responses were received from the students in the first year (31.4%) and final year (8.5%) respectively (Table 1).

Table 1: Demographic details of participants

Batch and Year	N (%)
Batch	
Males	158 (47.7%)
Females	173 (52.3%)
Year of study	
First	104 (31.4%)
Second	41 (12.4%)
Third	68 (20.5%)
Fourth	47 (14.2%)
Fifth	43 (13)
Final	28 (8.5%)

Most students (63.1%) used smartphones for online education, followed by tablets (60.7%), laptops (44.4%) and desktop computers (2.7%). The majority spent between 4-8 hours daily on online education (58%), and 2-6 hours on online non-academic activities (56.2%) (Table 2).

Table 2: Average time spent daily during the COVID-19 pandemic on online education and non-academic activities.

Hours spent per day	Online education N (%)	Online non-academic activities N (%)
<2	6 (1.8%)	89 (26.9%)
≥2-<3.99	46 (13.9%)	100 (30.2%)
≥4-<5.99	94 (28.4%)	86 (26%)
≥6-<7.99	98 (29.36%)	37 (11.2%)
≥8-<9.99	49 (14.8%)	12 (3.6%)
≥10	38 (11.5%)	7 (2.1%)

The mean (±SD) time of going to bed was 1:07am (±2 hours 42 minutes), waking up was 7:11 am (±1 hour 32 minutes) and time to fall asleep was 37 (±84) minutes respectively. The number of hours of actual sleep ranged from 1-14 hours [mean (±SD)=6.4 (±1.4) hours] with the majority (59.5%) reporting <7 hours of sleep. 27.2% reported having <6 hours of daily sleep.

The mean (SD) global PSQI score was 6.21 (2.84). Poor sleep quality (PSQI score of ≥5) was reported in the majority (71.3%). The mean PSQI score was ≥5 in students of all six batches, with the highest (7.18) and the second highest (6.91) mean scores reported among the students of the final and first years respectively (Table 3).

Table 3: Mean ± SD of PSQI* components, global scores and prevalence of poor sleep quality among medical students by year of study

Year of study	Sleep quality	Sleep latency	Sleep duration	Habitual sleep efficiency	Sleep disturbance	Sleep medication	Daytime dysfunction	PSQI Global Score	Poor sleep quality	
									N	%
1st	1.14 ± 0.69	1.04 ± 1.02	2.05 ± 0.93	0.29 ± 0.60	0.89 ± 0.59	0.14 ± 0.55	1.36 ± 0.74	6.91 ± 2.80	90	86.5
2nd	1.02 ± 0.61	1.00 ± 0.89	1.68 ± 0.99	0.10 ± 0.30	1.02 ± 0.52	0.07 ± 0.47	1.41 ± 0.71	6.32 ± 2.46	29	70.7
3rd	0.91 ± 0.64	1.09 ± 1.03	1.21 ± 1.06	0.26 ± 0.51	0.87 ± 0.54	0.04 ± 0.21	1.22 ± 0.69	5.60 ± 2.94	42	61.8
4th	0.94 ± 0.64	1.26 ± 0.92	0.85 ± 0.93	0.17 ± 0.43	0.87 ± 0.54	0.23 ± 0.73	1.36 ± 0.85	5.68 ± 3.01	27	57.4
5th	0.81 ± 0.50	0.88 ± 0.91	1.33 ± 0.99	0.12 ± 0.32	0.81 ± 0.50	0.09 ± 0.37	1.28 ± 0.70	5.33 ± 2.17	28	65.1
Final	1.04 ± 0.58	1.00 ± 0.94	2.21 ± 0.79	0.54 ± 1.04	0.82 ± 0.67	0.21 ± 0.63	1.36 ± 0.78	7.18 ± 3.24	20	71.4
Total	1.00 ± 0.64	1.05 ± 0.97	1.58 ± 1.06	0.24 ± 0.56	0.89 ± 0.56	0.13 ± 0.51	1.33 ± 0.74	6.21 ± 2.85	236	71.3

*PSQI- Pittsburgh sleep quality index

The majority complained of sleep problems due to inability to fall asleep within 30 minutes (58.6%) or

having bad dreams (46.5%). 24 students (7.2%) had used sleep medications at least once in the past month. (Table 4).

Table 4: Responses to individual PSQI questions on factors affecting sleep, use of medications to help sleep and trouble staying awake during activities*.

	Not during the past month		Less than once a week		Once or twice a week		≥3 times a week	
	N	%	N	%	N	%	N	%
How often have you had trouble sleeping because you...								
Cannot get to sleep within 30 minutes	137	41.4	89	26.9	52	15.7	53	16
Wake up in the middle of the night or early morning	186	56.2	69	20.8	57	17.2	19	5.7
Have to get up to use the bathroom	212	64.0	71	21.5	36	10.9	12	3.6
Cannot breathe comfortably	275	83.1	35	10.6	17	5.1	4	1.2
Cough or snore loudly	278	84.0	29	8.8	20	6.0	4	1.2
Feel too cold	221	66.8	59	17.8	41	12.4	10	3.0
Feel too hot	216	65.3	64	19.3	40	12.1	11	3.3
Had bad dreams	177	53.5	88	26.6	46	13.9	20	6.0
Have pain	243	73.4	48	14.5	33	10.0	7	2.1
How often have you taken medicine to help you sleep?	307	92.7	12	3.6	6	1.8	6	1.8
How often have you had trouble staying awake while driving / eating / social activity?	202	61.0	70	21.1	46	13.9	13	3.9

*All questions were limited to the preceding 4 weeks

The proportion of students who had taken sleep medication was highest in the final year [4 of 29 respondents (13.8%)]. However, there was no statistically significant association of such medication use among the batches. A total of 129 (38.5%) had trouble staying awake while driving, meals, or social activity at least once during the past month, being the highest amongst the students in the first year [48 of 105 9 45.7%)] followed closely by final year [13 of 29 (44.8%)] though there was no statistically significant association between the batches.

Presence of poor sleep quality was significantly associated with the batch ($p<0.05$) with significantly higher prevalence of poor sleep among first year students than third- and fourth-year students. Poor sleep quality had a significant positive association with hours spent on online education ($p<0.05$) and the use of laptops ($p<0.05$) but not with use of other types of devices. Sleep quality showed no associations with gender, examination scores and hours spent on non-academic online activities.

DISCUSSION

This study reveals a high prevalence (71.3%) of poor sleep quality and evaluates the associated factors among medical undergraduates undergoing online medical education amidst the COVID-19 pandemic hitherto not reported in Sri Lanka.

In a similar pre-pandemic study among medical students of the Faculty of Medicine and Allied Sciences of the University of Rajarata in 2017, 38.6% had poor sleep quality (global PSQI score ≥5) [9]. Globally, poor sleep quality was reported

among medical students in similar studies from India (39% of 504) [10], USA (50.9% of 314) [11] and Nepal (44.2% of 217) [12]. During the COVID-19 pandemic, poor sleep quality was reported among 30.4% of 168 Nepalese medical undergraduates with a mean (SD) PSQI score of 4.24 (2.19). [13]. In our study, poor sleep quality was found to be almost double compared to pre-pandemic studies by Ekanayake, Gunathilaka (9) and Shrestha, Adhikari (13). The mean (SD) PSQI score of 6.21 (2.84) was also higher compared to the study by Shrestha, Adhikari (13).

A significant increase from pre-pandemic to post-pandemic prevalence of poor sleep quality (58%-73%) and (mean (SD) global PSQI score [5.37 (3.01)-6.97 (3.54)] were reported among 307 university students in Italy [14]. Our study during the pandemic shows comparable results with 71.3% prevalence of poor sleep quality. The exponential increase in the number of online teaching-learning hours with the shift to online education during the COVID-19 pandemic could explain the increasing prevalence of poor sleep quality from pre-to post pandemic studies.

A study of medical students in Saudi Arabia during the early COVID-19 pandemic revealed that 73.8% of medical students complained of at least one sleep disorder [15]. The current study was not designed to assess distinct sleep disorders, however, it notable that the rates of sleep disorders in study by Abdelmoaty Goweda, Hassan-Hussein (15) is comparable to the high prevalence of poor sleep in the current study. This highlights the need for further studies to assess possible reasons for such high prevalence and the existence of sleep disorders.

The findings of the current study found no statistically significant association in sleep quality with gender as in studies by Brick, Seely (11) and Marelli, Castelnuovo (14). However, significantly higher odds of poor sleep quality (odds ratio 2.25, confidence interval 1.14-4.43) was seen among female medical students by Shrestha, Adhikari (13) and this difference persisted with adjustment for age and year of study. Female medical students had significantly poorer sleep quality in the Indian study by[10]

The prevalence of poor sleep quality in our study was significantly higher among first year students than third- and fourth-year students. A study of 860 US medical students reported higher rates of sleep-related problems in first and third year students than second and fourth year students, with sleep somnolence being highest in first and second year students [16]. Shrestha et al., 2021 reported poorer age and gender-adjusted sleep quality in fourth year than second year students [13].

The present study also demonstrated a statistically significant association of poor sleep quality with hours spent on online education and laptop device usage. Device/screen usage has been shown to cause sleep disturbances both in adolescents and adults [4, 5]. However, the present study failed to show a significant association between hours spent on non-academic online activities and with usage of devices other than laptops warranting further studies to identify possible reasons for these differences.

Majority (59.5%) of medical students in the current study did not have the 7 or more hours of nightly sleep recommended for healthy adults by American Academy of Sleep Medicine and Sleep Research Society [1] and endorsed by the Centre for Diseases Control and Prevention. The pre-pandemic study by Ayala, Berry (16) reported an average of 6.85 hours of actual sleep (range 3-10 hours) per night, similar to the mean (6.4 hours) and range (1-14 hours) reported in the present study. A study in Nepal during the pandemic reported a mean (SD) sleep duration of 7.5 (1.4) hours [13]. However, the average self-reported sleep duration of Sri Lankan adults was reported to be relatively short with a mean (SD) of 6.4 (1.5) hours [17], comparable to the average sleep duration of medical students in our study.

The proportion of students who had used sleep medication was higher in the present study (7.2%) compared to zero [13] and 6% [12] in previous studies. The larger proportion of students taking medications may suggest a higher use of over-the-counter medications or higher prevalence of diagnosed sleep disorders which need further evaluation.

Limitations of the current study include the inability to compare pre- and post-pandemic sleep quality, and the degree of influence of online education on sleep. Further studies are required to provide more definite conclusions regarding the

impact of online medical education on poor sleep quality and its outcomes.

CONCLUSION

Poor sleep quality was highly prevalent among medical undergraduates undergoing online medical education during the COVID-19 pandemic, especially first year students, and significantly associated with the time spent on online education and use of laptops. This may have adverse physical and mental health consequences and needs prompt investigation and action especially when designing online curricula.

Author declaration
Author contributions:
All authors were involved in the initial conception of the study and planning of methodology. P. C. Yasawardene and A. S. De Silva contributed to data collection. All three authours contributed to data analysis and drafted the manuscript. All authors edited and revised the final manuscript.

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Statement on Data availability on request from corresponding author:
Data is available on request from corresponding author.

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Original Research

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Prediction of respiratory failure in patients hospitalized with self-ingestion of organophosphorus insecticides

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ABSTRACT

Introduction: Self-poisoning with organophosphorus insecticides (OPI) is a global problem with most numbers being reported in the developing world. This is associated with a high morbidity including respiratory failure. However, studies on clinical prediction of respiratory failure are limited apart from a few retrospective studies.
Methods: A three-year prospective cohort study was conducted in a tertiary care hospital in Sri Lanka to identify the possible clinical predictors of respiratory failure. Basic demographic data, on-admission clinical parameters and red blood cell acetylcholine esterase levels (AChE) were recorded on patients who were suspected with OPI poisoned patients. Two separate analyses were conducted to assess the predictors of RF that occurred within 24 hours from ingestion of the poison (RF<24) and RF that occurred 24 hours after (RF>24). Variables that were differently distributed among patients who did and did not develop RF in each instance were retained for model building ($p< 0.25$ in univariate logistic regression analysis). Manual backward stepwise regression was used to develop two multiple logistic regression models separately for RF<24 and RF>24 using STATA 9.0 (StataCorp. 2005. *Stata Statistical Software: Release 9*. College Station, TX: StataCorp LP) software.
Results: Decreasing on-admission Glasgow coma scale (GCS) (odds ratio =0.57, 95% confidence interval 0.45-.073, p=0.000) and increasing pulse rate (odds ratio=1.03, 95% confidence interval 1.00-1.05, p=0.017) recorded on admission was predictive of RF occurring within 24 hours from ingestion of an OPI. GCS score of <13 were predictive of respiratory failure which occurred within the first 24 hours with a sensitivity of 60% and a specificity of 90.7%. The area under the curve (AUC) for the receiver operating characteristic (ROC) curve drawn for GCS was 0.77 which shows a moderate predictive ability. AUC of the ROC curve drawn for pulse rate was 0.67 indicating its poor predictive ability.
Conclusion: On-admission GCS is a good tool with a moderate sensitivity to triage those who are at risk of developing respiratory failure within 24 hours following OPI ingestion.

INTRODUCTION

Self-poisoning by organophosphorus insecticides (OPI) is a problem which mainly affects the developing world [1-6]. Despite the regulation on highly toxic pesticides [7-9], ingestion of OPIs are still associated with a high morbidity including respiratory failure (RF) [1, 3, 10-15].

RF that is seen in OPI ingestion is classically described to be associated either with the initial cholinergic crisis or the intermediate syndrome (IMS) [11, 15-17]. The cholinergic crisis is seen in the initial post ingestion period due to predominant muscarinic receptor overstimulation. Commonly seen features include bradycardia, hypotention, lacrimation, urinary incontinence and excessive sweating [11, 12, 18-20]. Effects on the respiratory system include cough, rhinorrhea, bronchorrhea, bronchospasm, leading to severe respiratory distress and probable subsequent ventilation. Central respiratory depression and respiratory muscle weakness are the key features associated with severe respiratory distress in patients whose cholinergic effects have otherwise been overcome by atropine and oxygen therapy [13, 16].

RF seen in IMS as first described in 1987, usually sets in after the initial cholinergic features had waned off, between 24-96 hours post-ingestion[21]. The mechanism of RF is attributed to nicotinic receptor overstimulation and subsequent blocking at the neuromuscular junction leading to respiratory muscle paralysis. These patients show minimal cholinergic features in the initial period after ingestion [22-24].

Most of the patients who develop RF following ingestion of an OPI are from rural areas of the country where the first encounter is a primary care hospital with limited resources. One of the key areas of management is the early identification of patients who are at risk of possible RF. However, an easily administrable validated clinical tool is not available to identify such patients and triage them for ventilator care [3, 17]. Although a few studies had addressed this issue, those were retrospective cohort studies based on Intensive care unit (ICU) records and scoring systems rather than on prospective data [13, 25, 26]. Therefore, we conducted this prospective cohort study in a tertiary care hospital to investigate the possibility of predicting the RF associated with OPI poisoning to facilitate the selection process of high-risk patients of RF based on their on-admission parameters to monitor and triage them into limited ventilator and ICU facilities available.

METHODS

A prospective cohort study was carried out on patients with acute OPI self-poisoning at Teaching Hospital Peradeniya (THP) located in the Central Province of Sri Lanka, a tertiary care referral centre with a specialized toxicology ward and an ICU for management of insecticide poisoned patients. Ethical clearance to this study was obtained from the Human Research Ethics Committee of the University of Peradeniya, Sri Lanka (2012/EC/63).

Patients with a history of self-ingestion of an OPI were seen on admission and data were recorded prospectively from 1st of March 2013 to 30th of September 2016. The poison ingested was provisionally identified from the patient's or relative's history or based on the bottles provided to the hospital or based on the transfer documents and accompanying doctor's comments. Additionally a blood sample was collected and red blood cell acetylcholine esterase inhibition was estimated using Test-mate ChE (EQM Research, Inc., Cincinnati, OH) kit which has been validated as being accurate by comparison to samples stored and sent to a reference laboratory in Munich [27-29].

Written informed consent to be recruited into the study was obtained from the patient or next of kin. Patients who were below16 years of age and those who were pregnant were excluded. Also, patients who were already intubated while being admitted to THP and those who required intubation within 2 hours from admission were excluded (The latter group of patients had a GCS=<8 and as GCS <8 was an indication to intubate, these patients were excluded from the analysis). Patient recruitment flow chart is given in figure 6.1.

All the patients were examined on admission for cholinergic features such as sweating, pinpoint pupils, urinary or fecal incontinence, bi-basal crepitations for any signs of bronchorrhoea and bronchospasms and hypotension apart from all the

vital parameters while being treated according on a standard protocol [30]. Some patients required resuscitation and intubation on admission. All the patients with cholinergic features were intravenously atropinized starting from an initial dose of 10-15 mg until the patients were clinically stabilized and until bronchorrhoea and bronchospasms had resolved. Thereafter a continuous infusion of atropine in 0.9% normal saline was set to keep the patient free of cholinergic features.

Consistency of the clinical parameters that were observed was maintained by objective assessment through a structured questionnaire. Patients were assessed twice daily in the ward aside the frequent visits in the initial period. All the events that took place with regard to a patient's condition were noted by the study doctors.

Management of the patients was based on protocols agreed between the ward doctors and the study team. Decisions related to intubation and transfer of the patients into the ICU was made independently by the medical team based on the clinical condition of the patient irrespective of the type or the quantity of the OPI the patient had ingested. Patients were paralyzed and sedated with atracurium or midozolam in order to be intubated. In the cohort the need for intubation was considered to be synonymous with RF.

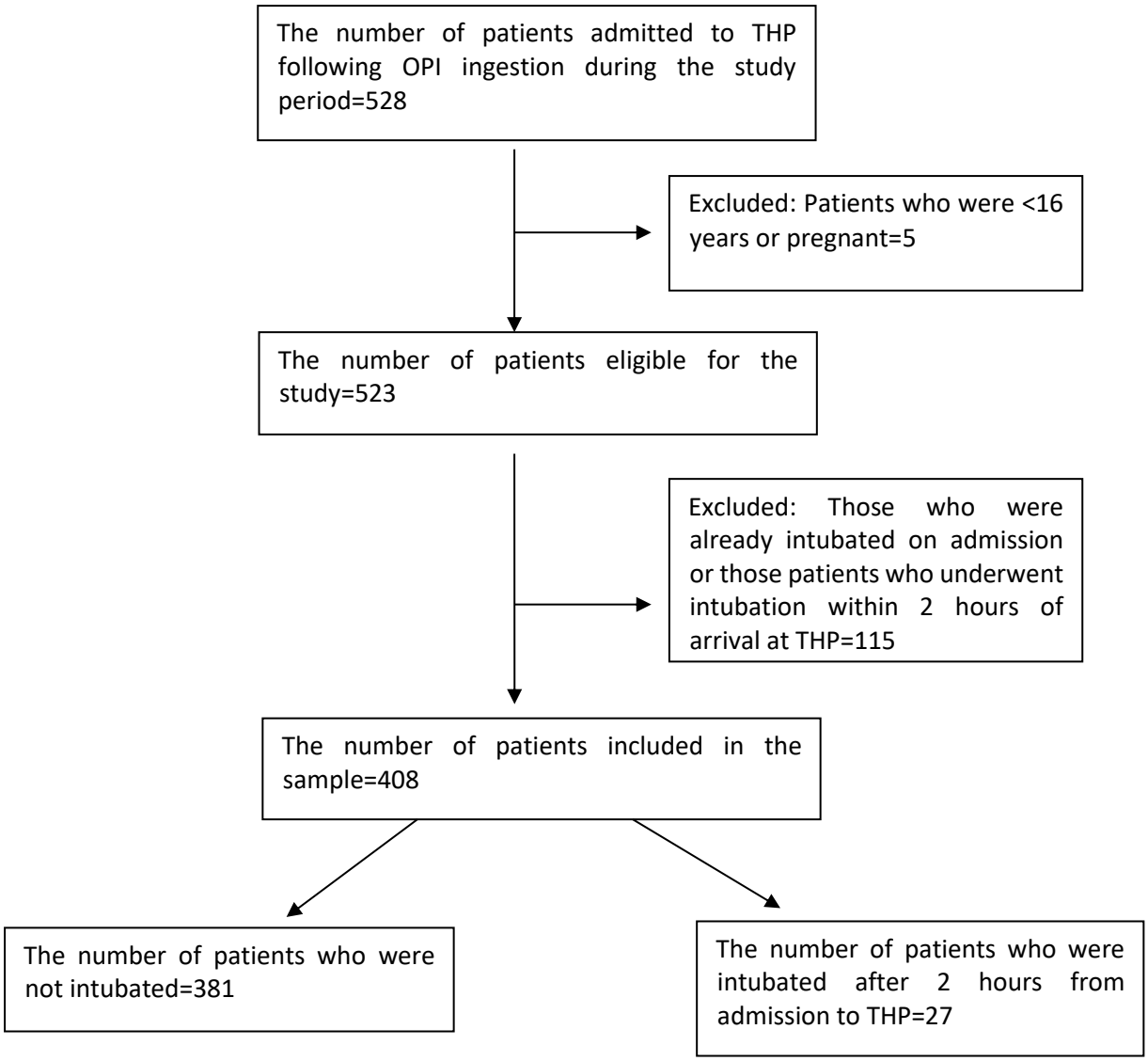

Figure 1: Recruitment flow chart of patients

Data Analysis

The primary data analysis was done using PRISM, version 5 (Graph Pad Software, Inc., La Jolla, CA). Conceptual framework and the factors considered for the multiple regression model building are summarized in figure 2. Characteristics of continuous variables (age, systolic blood pressure, diastolic blood pressure, pulse rate, respiratory rate) were reported as means, and 95% confidence intervals (95% CI). Highly skewed continuous variables (miosis and red blood cell acetylecholine esterase levels) were reported as median and inter quartile ranges (IQR). Nominal variables (gender and type of the OPI ingested by the patient) and binominal ordinal variables (alcohol status, and presence or absence of fasciculation) were summarised as percentages. On admission GCS score was also summarised as a percentage.

Two separate analyses were conducted to assess the predictors of RF that occurred within 24 hours from ingestion of the poison; excluding those who required intubation within 2 hours from admission (RF<24) and RF that occurred 24 hours later from ingestion of the insecticide (RF>24). The reason for such division was based on the differences in pathophysiology of RF in those two instances. The RF<24 is attributed to depression of the central respiratory drive and excessive bronchial secretions[16] while the RF>24 is attribute to be associated with IMS following nicotinic receptor blocking at the neuromuscular junction[21-23].

The primary outcome of the first analysis was RF within 24 hours or not. The primary outcome of the second analysis was RF that occurred after 24 hours from ingestion of the poison or not. Variables that were differently distributed among patients who did and did not develop RF (In the first instance RF< 24 hours and the second instance RF>24hours) were retained for model building ($p< 0.25$ in univariate logistic regression analysis). There was no significant co linearity between the possible predictors ($r =>0.5$). Manual backward stepwise regression was used to develop two multiple logistic regression models separately for RF<24 and RF>24 using STATA 9.0 (StataCorp. 2005. *Stata Statistical Software: Release 9*. College Station, TX: StataCorp LP) software.

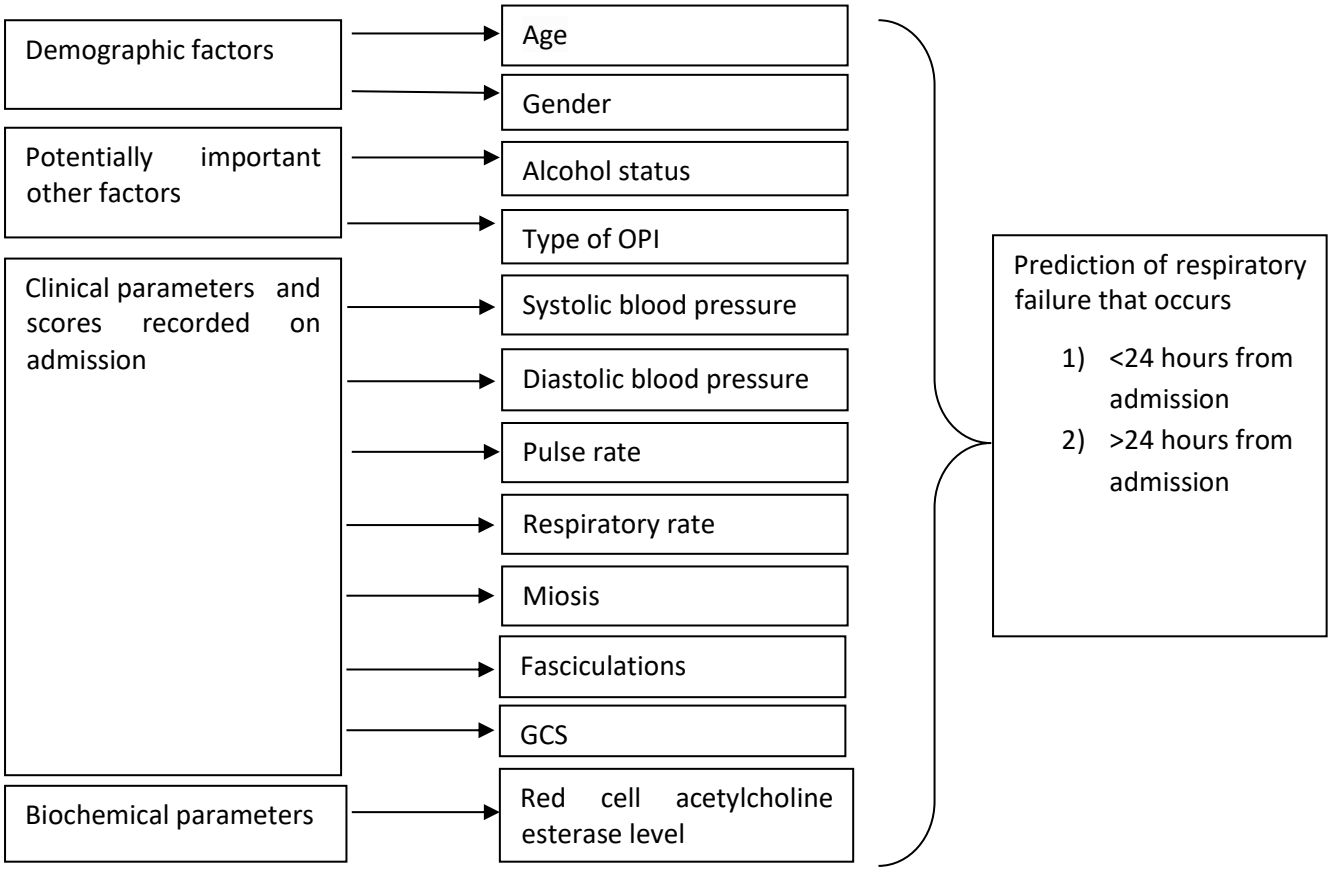

Figure 2: Conceptual framework of the factors that were considered for predicting RF.

RESULTS

During the study period 528 patients who had ingested an OPI compound was admitted to Teaching Hospital Peradeniya. Based on our criteria 408 patients were recruited to the study. The patients were divided into three groups: those who did not develop respiratory failure (RF-), those who developed RF within 24 hours from ingestion of the insecticide (RF<24) and those who developed RF afterwards (RF>24). The characteristics of those patients are given in table 6.1.

Table 1: Characteristics of patients who ingested OPIs.

Variable	**RF- (n=381)**	**RF<24(n=22)**	**RF>24(n=5)**
Age: mean (95% CI)	37.1(35.58-38.65)	44.5(38.9-50.0)	47.4(29.31-65.49)
Males: number (%)	283(74%)	20(91%)	5(100%)
Alcohol Consumed: number (%) Status unknown: number	 112(34%) 51	 15(71%) 1	 1(20%) 4
Type of OPI Chorpyrifos: number (%) Profenofos: number (%) Phenthoate: number (%) Diazenon: number (%) Unknown: number (%)	 87(23%) 70(18%) 10(3%) 36(9%) 157(41%)	 3(13%) 3(13%) 2(9%0 2(9%) 14(63%)	 0 2(40%) 0 0 3(60%)
Systolic blood pressure: mean (95% CI)	117.7(116.3-119.2)	121.5(115.2-127.9)	110.8(89.2-132.4)
Diastolic blood pressure: mean (95% CI)	74.84(73.8-75.8)	79.55(75.1-84.0)	67.8(49.8-85.7)
Pulse rate: mean (95% CI)	79.98(78.5-81.4)	96.18(83.6-108.8)	83.8(61.3-106.3)
Respiratory rate: mean (95% CI)	18.72(18.2-19.20)	18.05(15.9-20.1)	15.6(12.3-18.8)
Miosis: median (IQR)	3(3-3)	3(2-3)	3(1.5-3)
Fasciculations Present: number (%) Unknown: number	 20(8%) 134	 1(6%) 5	 1(25%) 1
Red blood cell acetylcholine esterase in units per gram of haemoglobin): median (IQR)	0.74(0-17.68)	2.54(0-10.39)	11.8(0-26.7)
GCS: median (IQR) Score 15: number (%) Score 14: number (%) Score 13: number (%) Score 12: number (%) Score 11: number (%) Score 10: number (%) Score 9: number (%)	15(15-15) 336(88%) 11(3%) 14(4%) 7(2%) 4(1%) 3(<1%) 5(1%)	11(10-15) 8(36%) 1(4%) 1(4%) 1(4%) 3(14%) 4(18%) 4(18%)	14(13-14) 2(40%) 2(40%) 0 1(20%) 0 0 0

Multiple logistic regression analysis of those who developed respiratory failure within 24 hours

In this model p= 0.000, pseudo R^2 =0.33 with a log likelihood of -53.30 (Table 2). Overall model was significant.

Table 2: Multiple logistic regression model for prediction of respiratory failure that occurred within 24 hours.

	Odds Ratio	**95% Confidence Interval**	**P value**
Age	1.03	0.99-1.07	0.10
Gender	0.73	0.11-4.83	0.74
Alcohol status	3.57	0.90-14.09	0.069
Systolic Blood Pressure	0.97	0.92-1.04	0.38
Diastolic Blood Pressure	1.05	0.97-1.15	0.19
Pulse rate	**1.03**	**1.00-1.05**	**0.017**
GCS	**0.57**	**0.45-0.73**	**0.000**

Footnote: Bold indicates statistical significance.

Out of all factors pulse rate and GCS recorded on admission of the patient into THP became significant in predicting respiratory failure occurring within 24 hours following ingestion of an OPI.

According to the model increasing pulse rate was predictive of respiratory failure. However, the area under the curve (AUC) in the receiver operating characteristic curve (ROC) drawn for pulse rate was 0.67 indicating its poor ability to predict RF<24.

An increase in a single score of GCS was 1.75 times more protective of the patient from being ventilated following RF within 24 hours of ingestion of an OPI. AUC for the ROC drawn for GCS was 0.77 with a sensitivity of 60.0% and a specificity of 90.67% for a GCS <13 indicating its moderate predictive ability of probable RF within 24 hours from ingestion.

Multiple logistic regression analysis of those who developed respiratory failure after 24 hours.

In this model p=0.075, pseudo R^2= 0.55 with a log likelihood of -5.33 (Table 6.3). Thus, the overall model was not significant.

In this group of patients none of the factors that were considered in the model could predict RF that occurred 24 hours after from ingestion of the OPI.

Table 3: Multiple logistic regression model for prediction of respiratory failure that occurred after 24 hours.

	Odds Ratio	**95% Confidence Interval**	**P value**
Age	1.00	0.96-1.03	0.98
Profenofos	26.12	0.03-20933	0.33
Systolic blood pressure	1.00	0.67-1.48	0.99
Diastolic blood pressure	0.78	0.51-1.17	0.24
Respiratory rate	0.938	0.83-1.06	0.31
Pupillary size	0.02	0.00-25.92	0.28
Fasciculations	526	0.04-6888935	0.19

DISCUSSION

RF is one of the commonest reasons behind the high mortality associated with OPI ingestion [1, 2, 4, 15, 31]. Therefore, we focused on predicting RF based on prospectively collected data of over 400 patients. We conducted separate analyses to predict RF occurring within 24 hours following ingestion of the OPI; attributed to cholinergic crisis and RF which occurred between 24-96 hours; attributed to IMS. The pathophysiology of RF associated with cholinergic crisis is thought to be due to depression of the central respiratory drive and blockage of the respiratory tract following secretions [11, 32] and the RF associated with IMS is thought to be due to respiratory muscle blockage at the neuromuscular junction[22].

In the group of patients who developed RF within 24 hours from ingestion of the OPI, on admission pulse rate and GCS score were predictive of RF. The mean pulse rate of the group of patients who did not develop RF was 79.98 and the mean pulse rate of the group of patients who developed RF within 24 hours was 96.18. Usually bradycardia is seen in patients who develop cholinergic crisis [32] following OPI ingestion. However, in our study we found a higher pulse rate to be more predictive of RF associated with the cholinergic crisis which occurs within 24 hours from ingestion of the poisoning (OR=1.03). This high pulse rate recorded could be related to the anxiety or distress in the patient following OPI ingestion in the initial period. A few earlier studies also reported of a tachycardia associated with OPI poisoning in the initial period especially in children[33-35] In our study even though there was a significant difference between the mean pulse rates of the two groups, both were within the normal range of pulse rate in a human which is between 60-100 beats per minute [36]. The ROC curve drawn for pulse rate for predicting RF <24 had a very poor predictive ability with an AUC of 0.67 [37]. Therefore, it becomes difficult to predict RF in a patient following OPI ingestion even though the patient could be having a high pulse rate.

We also found that an increase in 1 score of GCS score was 1.75 times more protective of the patient going into RF within 24 hours from ingestion of the poison (OR for GCS being 0.57).The AUC for the ROC cure drawn for GCS was 0.77 which showed the moderate predictive ability of GCS score in predicting RF occurring within 24 hours from ingestion of the OPI [37]. This could be predicted with a 60% sensitivity and a 90.7% specificity for a patient who presented with a GCS <13. This is consistent with a previous such study done in India where GCS was helpful in predicting RF [13]. However, this study mainly focused on RF associated with IMS. In contrast, in our study we could not find any predictors of RF associated with IMS which usually sets in after 24 hours from ingestion of the poison. Based on these findings as GCS score is a very versatile tool that can be applied in the initial period by medical or para-medical personals, it can be used to triage and monitor patients with a higher risk of developing RF within 24 hours and transfer them into hospitals where ventilator facilities are available.

A previous study report advancing age to be associated with cholinergic RF [38] but in our model age did not become a predictor of RF. We observed a male predominance in the RF similar to what was observed in the same region [5, 39-41] though it also did not become a predictor. Co-ingestion of alcohol was reported to be common among those who develop RF in OPI ingestion [42, 43]. Despite the fact that 71% of patients who developed RF within 24 hours were under the influence of alcohol, we did not find ingestion of alcohol to be a predictor of RF as well. In both non respiratory failure and RF<24-hour groups the respiratory rate was around 18.

In contrast to the group that developed RF < 24 hours none of the parameters could predict RF that developed 24 hours later from ingestion of the poison. As mentioned earlier, a previous study done in India reports GCS to be a predictor of IMS and RF associated with it [13]. However, in our study we observed that profenofos and fasciculations had significnaly high OR associated with very wide confidence intervals. The reason why they did not become significant could be due to the small sample size of patients. Further on, the number of patients whose type of poison was unknown is high. If we were to know the type they have ingested, and if a significant number in the late RF group were to have ingested profenofos maybe it too would have become a significant factor associated with delayed RF.

Fasciculations are involuntary muscle contractions which occur following complete blocking or de-

innervation of the muscle due to failure of impulse conduction across the neuromuscular junction [44]. In our study we thought this could become a predictor of RF failure associated with IMS as the underlying pathophysiology of RF is similar in nature and is attributed to neuromuscular junctional blocking in both instances [45]. However, this did not become a predictor of RF occurring >24 hours from ingestion of the poison. The reason may be related to the small sample size of the patients who developed RF >24 hours following ingestion of OPI.

One major limitation of our study was the late patient presentation to Teaching Hospital Peradeniya as it is a tertiary care referral centre. This led to omission of patients who were already intubated and those who required ventilation within 2 hours of admission, which reduced the number of patients with respiratory failure included in the analysis. Another limitation of our study was that the type of OPI ingested by all patients was not known. However, with the available numbers none of the different OPIs became a predictor of RF.

CONCLUSION

GCS can be used as a versatile tool to triage and closely monitor those who are at risk of developing RF within 24 hours from ingestion of the poison. A GCS score of <13 is predictive of RF failure occurring within 24 hours with 60% sensitivity and 90.7% specificity.

Author declaration
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Disclosure statement
The authors have no conflicts of interest to disclose.

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Pulmonary functions of adult women primary cooks in Colombo District, Sri Lanka: A cross sectional study

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ABSTRACT

Background: There are growing concerns about the deleterious effects of cooking fuel smoke on pulmonary functions.
Objectives: To determine the pulmonary functions of women primary cooks in the Colombo district and its association with the cooking fuel used.
Method: A descriptive cross-sectional study was carried out among adult women primary cooks recruited using a two-stage cluster sampling method (n=600). Their baseline data, data on cooking fuel use, smoking status and exposure to other air pollutants were obtained using an interviewer-administered questionnaire. Pulmonary functions (FEV_1, FVC, FEV_1/FVC ratio, $FEF_{25\%-75\%}$) were assessed using spirometry (COSMED Pony Fx, Italy) according to ERS/ATS guidelines. Data analysis was done using SPSS version 23 and a p-value<0.05 was considered significant.
Results: Mean age of women was 45.5 years ±14.2SD with mean of 5.4 hours of cooking per day ±2.0SD and a mean 24.8 years of cooking ±14.5SD. Among them, 65% used biomass fuel and the remaining 35% used liquid petrol gas. FEV_1 (1.71L ±0.50SD vs. 1.94L ±0.47SD), FVC (1.97L ±0.55SD vs. 2.21L ±0.53SD) and $FEF_{25\%-75\%}$ (2.12L/s ±0.84SD vs. 2.40L/s ±0.82SD) were significantly lower in women using biomass fuel compared to women using liquid petroleum gas (p<0.05). However, only FEV_1 showed a significant association with the cooking fuel (β=-0.066, p<0.05).
Conclusion: The use of biomass fuel for cooking was significantly associated with a decline in pulmonary functions and a higher prevalence of obstructive airway disease. Thus, appropriate measures should be taken to promote the use of clean cooking fuel among women in Sri Lanka to reduce the pulmonary dysfunctions associated with the use of biomass fuel for cooking.

Keywords: Pulmonary functions, cooking fuel, Airway obstruction, Indoor air pollution, Household air pollution

INTRODUCTION

Indoor air quality has a significant impact on people's health and some studies have shown that the level of indoor air pollutants is greater than that of outdoor settings (1). Household activities such as smoking, combustion, and cleaning contribute to poor indoor air quality (2). The choice of household cooking fuel depends mainly on socioeconomic conditions (3). Those who have poor socioeconomic status use biomass fuel which is available as a free commodity. Three billion people worldwide use solid fuel for cooking (4). Most of them are aggregated in Southeast Asia and Africa, in the regions where over 60% of households use solid fuel for cooking (5,6). The latest data in Sri Lanka in 2016 showed that 66% of the population uses biomass fuel for cooking with 24.9% in urban, 73.4% in rural and 79.9% in estate sectors (7). This shows a 12.5% drop in the use of biomass fuel from 2007 to 2016 but unfortunately, this declining trend has changed recently as Sri Lanka undergoes a major economic crisis in 2022. The authorities failed to maintain an uninterrupted supply of liquid petroleum gas. Further, the price of a domestic liquid petroleum gas cylinder has increased almost by 50%. Thus, the lack of availability and the price hike has now limited the use of liquid petroleum gas for domestic cooking by Sri Lankan people who are affected by the inflation surge making them more vulnerable to the deleterious effects of cooking fuel smoke. However, the exact figures on the increase in the use of biomass fuel and kerosene during this period of the economic crisis are not currently available but are expected to be high.

Biomass is any recently living plant or animal material that is burnt as fuel. However, burning biomass in low-efficiency stoves generates air pollutants such as particulate matter, ozone, sulphur dioxide, nitrogen dioxide, and carbon monoxide that are known for their toxic effects on airways (8,9). In Asian countries, women being the primary cooks of the households are most heavily exposed to cooking fuel smoke than the other family members making them more vulnerable to pulmonary diseases. About 57% of chronic obstructive pulmonary disease (COPD) patients in Sri Lanka were reported to be non-smokers which is another indication of the need of assessing other possible contributory factors for COPD such as biomass fuel smoke exposure (10). Globally, indoor air pollution due to cooking fuel smoke is studied in detail as an emerging public health concern and studies in other countries have shown that exposure to biomass fuel smoke was associated with a broad range of respiratory outcomes which include increased respiratory symptoms, impaired respiratory functions, increased risk of COPD, and acute lower respiratory tract infections (6,11–14). This broad range of respiratory outcomes of biomass fuel smoke exposure is known to influence by age, individual sensitivity, cultural practices, lifestyle, and socioeconomic status (15,16). Further, exposure to cooking fuel smoke depends mainly on three factors; Source of pollution, living environment and User behaviours and some of the features related to these are unique to Sri Lanka. Therefore, the results of the studies from other countries cannot be directly generalized to Sri Lanka.

It is well known that exposure to biomass fuel smoke is associated with poor lung functions. However, the results of the Burden of Obstructive Lung Disease (BOLD) showed conflicting evidence and concluded that the airflow obstruction was not associated with the use of solid fuels for cooking or heating (17). To our surprise, except for the BOLD study, there were no studies conducted to assess pulmonary functions of biomass fuel users in Sri Lanka even if it is commonly used. Therefore, there is a need for a more focused study on women in Sri Lanka to assess the pulmonary functions based on cooking fuel use. Thus, the present study was carried out aiming to assess pulmonary functions of women primary cooks in selected areas of the Colombo district, Sri Lanka, and its association with cooking fuel use.

MATERIALS AND METHODS

Study design and setting

A community-based descriptive cross-sectional study was conducted among women primary cooks (n=600) residing in Hanwella and Padukka Medical Officers of Health areas of Colombo District, Sri Lanka from October 2017 – September 2018.

Study population

The participants were adult women primary cooks residing in randomly selected households in selected Grama Niladari Divisions of Hanwella and Padukka Medical Officers of Health areas of Colombo District, Sri Lanka who met the inclusion/exclusion criteria. The inclusion criteria were women primary cooks ≥18 years of age who are residing at the selected household and who have been cooking for at least last one year. The exclusion criteria were those who were currently pregnant, disabled, had recent (last three months) chest injuries, chest surgeries, or eye surgeries and diagnosed with heart diseases.

Sample size

The required sample size was calculated using statistical formula (18) assuming a prevalence of COPD of 10% in female primary cooks (10). For 80% power at a 5% significance level, a dropout rate of 10%, and a design effect of 3.9 (19,20), a sample size of 600 was needed for the study.

Sampling method

Two-stage cluster sampling technique was employed to select the study participants. At the stage one of cluster sampling, as primary sampling units, twenty (20) Grama Niladari Divisions were selected from a list of eighty-six (86) Grama Niladari Divisions of Hanwella and Padukka Medical Officers of Health areas by probability proportion to size method. In the stage two of cluster sampling, 30 households were randomly selected from each primary sampling unit by a household survey (19). The women primary cooks residing in the selected household who match the inclusion/exclusion criteria and available during the household survey were recruited in the study. Following this method, the total sample size of 600 women primary cooks was recruited.

Data collection

An interviewer-administered questionnaire was used to collect sociodemographic data, primary cooking fuel used, cooking practices, smoking status, exposure to other air pollutants such as secondhand smoking, occupational exposure to dust/smoke, industries in the vicinity emitting smoke/ dust, pet dog/cat at home and use of mosquito repellents. These were presented in elsewhere (21). A portable spirometer (Cosmed Pony Fx, Rome, Italy) was used for the assessment of pulmonary functions according to American Thoracic Society/ European Respiratory Society 2005 guidelines (22). The age, height and sex predicted values of forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), their ratio and maximum mid expiratory volume ($FEF_{25\%-75\%}$) were calculated using ethnic specific equation for Sri Lankan Sinhalese [36] and Sri Lankan Tamil [37] published in the literature. After verbal explanation and demonstration, the participant performed the forced vital capacity manoeuvre, without using a bronchodilator, in a standing position with the nose clip. The resulting volume-time and flow-volume curves were observed for the within manoeuvre acceptability criteria (22). After a few minutes of rest, when the participants were comfortable repeating the manoeuvres, another two technically acceptable manoeuvres were performed. Three technically acceptable manoeuvres were checked for repeatability criteria (22). From the successfully performed manoeuvres, the largest FVC and FEV_1 were determined. The below with the largest sum of FVC and FEV_1 was selected and stored to determine other indices for data analysis. Obstructive airway disease and restrictive airway disease were determined by the FEV_1 and FVC measured by spirometry based on the fixed cutoff values (25). The spirometry reports that did not show any of the abnormalities mentioned above were further screened using $FEF_{25-75\%}$. The $FEF_{25-75\%}$ provided information on small airway disease (26). Accordingly, the following cutoff values were used; Obstructive airway disease: FEV_1 ≤80% and FEV_1/FVC ratio ≤0.7,Rrestrictive airway disease: FVC ≤80% and FEV_1/FVC ratio >0.7 and Small airway disease: $FEF_{25-75\%}$ ≤65% (26,27).

Statistical analysis

Data was entered into the database and analysed by SPSS version 23. Each variable was evaluated for normality by graphical representation, Shapiro-Wilk test, and Kolmogrove test. For normally distributed variables, descriptive statistics were computed as mean ±SD (continuous variables) and frequencies (categorical variables). The women

were stratified into two groups based on the primary cooking fuel used: women using biomass fuel (W_{BMF}) and women using liquid petroleum gas (W_{LPG}). Independent sample t-test and one-way ANOVA with Tukey Post-Hoc test was used for mean comparisons. The Chi-square test was used to compare categorical variables. The associations were determined using the standardised β-coefficients computed using standard multiple linear regression analysis. The factors that had significant associations (r ≥0.3) or had a *p-value* ≤0.2 in univariate analysis were considered as potential predictor variables for regression analysis. *p-value <0.05* were considered significant.

RESULTS

Basic characteristics of women primary cooks

Out of the total 600 women primary cooks recruited, 94% (n=566) completed the study. The incomplete data of 34 subjects (due to not attending for the lung function testing and inability to complete the lung function tests) were not included in the final data analysis. The basic characteristics of women primary cooks are depicted in Table 1. The mean ±SD age of the women primary cooks was 45.5years ±14.2SD, within the age range of 20 -84 years. The W_{BMF} were significantly older than W_{LPG} (*p<0.05*). Their anthropometric parameters were significantly different from that of W_{LPG} (*p<0.05*). Further, W_{BMF} spent more time on cooking than the W_{LPG} (*p<0.05*). The smoking status and occupational exposures to air pollutants were low as 1% (n=6) and 2.1% (n=12).

Table 1 Basic characteristics of women primary cooks

Characteristics	Women primary cooks n=566	W_{BMF} n=366	W_{LPG} n=203	*p-value*
	Mean ±SD	Mean ±SD	Mean ±SD	
Age (in years)	45.5 ±14.2	47.2 ±14.4	42.5 ±13.2	*<0.001**
Height (cm)	151.6 ±6.5	150.8±6.3	153.0±6.8	*<0.001**
Weight (kg)	54.5 ±12.4	52.7±11.1	57.7±14.0	*<0.001**
BMI (kg/m^2)	23.8 ±6.7	24.3±7.8	22.8±4.2	*0.008**
Hours of cooking per day	5.4 ±2.0	5.9 ±2.1	4.6±1.4	*<0.001**
Years of cooking	24.8 ±14.5	29.3±14.4	16.7±11.9	*<0.001**

*W_{BMF}: Women using biomass fuel as primary cooking fuel, W_{LPG}: Women using liquid petroleum gas as primary cooking fuel, Those with kyphosis (n=5) were excluded, p-values obtained from independent sample t-test. *p<0.05 is significant.*

Pulmonary functions of women primary cooks

The pulmonary functions (FEV_1, FVC, FEV_1/FVC ratio and $FEF_{25\%-75\%}$) of women primary cooks are presented in Table 2. The W_{BMF} had significantly lower mean FEV_1 (1.71L ±0.50SD vs. 1.94L ±0.47SD), mean FVC (1.97L ±0.55SD vs. 2.21L ±0.53SD) and mean $FEF_{25\%-75\%}$ (2.12L/s ±0.84SD vs. 2.40L/s ±0.82SD) than W_{LPG} (*p <0.05*).

Table 2: Pulmonary functions of women primary cooks

	Women primary cooks n=561 Mean ±SD	W_{BMF} n=360 Mean ±SD	W_{LPG} n=201 Mean ±SD	*p-value*
FEV_1(L)	1.79 ±0.50	1.71 ±0.50	1.94 ±0.47	*<0.001**
FVC (L)	2.05 ±0.56	1.97 ±0.55	2.21 ±0.53	*<0.001**
FEV_1/FVC	87.58 ±9.01	87.42 ±10.01	87.87 ±6.69	*0.527*
$FEF_{25\%-75\%}$	2.22 ±0.84	2.12 ±0.84	2.40 ±0.82	*<0.001**

*W_{BMF}: Women using biomass fuel as primary cooking fuel, W_{LPG}: Women using liquid petroleum gas as primary cooking fuel, Those with kyphosis (n=5) were excluded, p-values obtained from independent sample t-test. *p<0.05 is significant*

Correlation of pulmonary functions of women primary cooks with age, anthropometric measurements, and cooking duration

The correlation coefficients for FEV_1, FVC, FEV_1/FVC ratio and $FEF_{25\%-75\%}$ of women primary cooks with age, anthropometric measurements and cooking duration are depicted in Table 3. FEV_1, FVC and $FEF_{25\%-75\%}$ had significant negative correlations with age and years of cooking (multicollinear relationship) and significant positive correlations with height and weight of W_{BMF} (*p<0.05*).

Table 3: Correlation of respiratory functions of women primary cooks with age, anthropometric measurements, and cooking duration

		FEV_1	FVC	FEV_1/FVC	$FEF_{25\%-75\%}$
	n	r	r	r	r
Age (in years)					
Women primary cooks	561	-0.66*	-0.66*	-0.02	-0.46*
W_{BMF}	360	-0.63*	-0.64*	-0.02	-0.45*
W_{LPG}	201	-0.68*	-0.67*	-0.01	-0.42*
Height (cm)					
Women primary cooks	561	0.53*	0.53*	0.03	0.40*
W_{BMF}	360	0.55*	0.56*	0.01	0.40*
W_{LPG}	201	0.46	0.43	0.07	0.34*
Weight (kg)					
Women primary cooks	561	0.32*	0.33*	0.00	0.26*
W_{BMF}	360	0.31*	0.30*	0.04	0.28*

W_{LPG}	201	0.26*	0.30*	-0.09	0.18*
BMI (kg/m^2)					
Women primary cooks	561	-0.06	-0.06	-0.02	-0.06
W_{BMF}	360	-0.05	-0.05	-0.02	-0.06
W_{LPG}	201	-0.001	0.01	-0.02	0.02
Hours of cooking per day					
Women primary cooks	561	-0.04	-0.06	-0.003	-0.02
W_{BMF}	360	0.02	0.02	-0.04	0.02
W_{LPG}	201	0.03	-0.03	0.16*	0.04
Years of cooking					
Women primary cooks	561	-0.61*	-0.61*	-0.02	0.42*
W_{BMF}	360	-0.63*	-0.64*	-0.02	-0.45*
W_{LPG}	201	-0.45*	-0.47*	0.02	-0.25*

*W_{BMF}: Women using biomass fuel as primary cooking fuel, W_{LPG}: Women using liquid petroleum gas as primary cooking fuel, those with kyphosis (n=5) were excluded, p-values obtained from Pearson correlation coefficient. *p<0.05 is significant*.

Association between pulmonary functions and cooking fuel used.

The regression analysis found out that the variations in the spirometry parameters can be predicted by age, height, weight, cooking fuel, having pet dog/cats at home and exposure to secondhand smoking. The results of the standard multiple linear regression analysis models are showed in Table 4. Accordingly, the above variables explained 56% of the variation of FEV_1 (R^2 = 0.56) and 56% of the variation in FVC (R^2 = 0.56) but, the same variables account only for 29% of the variation of $FEF_{25\%-75\%}$ (R^2 = 0.29). The normal probability plots and scatter plots of standardized residuals were used to assess the normality, linearity and outliers and found no violations of the assumptions of linear regression. The W_{BMF} had a reduction of FEV_1 of 0.066 L, FVC of 0.057 L and $FEF_{25\%-75\%}$ of 0.073 L compared to a W_{LPG} when controlled for the above variables but the result was significant only for FEV_1 (*p <0.05*).

Table 4: Standard multiple linear regression model for FEV_1, FVC and $FEF_{25\%-75\%}$ of women primary cooks

Variable	Coefficient	Standard error	*p-value*	95% CI
FEV_1				
Constant	-1.134	0.378	*0.003**	-1.876, -0.392
Age	-0.018	0.001	*<0.001**	-0.020, -0.016
Height	0.023	0.002	*<0.001**	0.018, 0.028
Weight	0.005	0.001	*<0.001**	0.002, 0.007
Use of BMF	-0.066	0.030	*0.031**	-0.125, -0.006

Having pet dog/cat	0.060	0.028	*0.034**	0.005, 0.116
Passive smoking	-0.050	0.033	*0.128*	-0.115, 0.015
FVC				
Constant	-1.060	0.418	*0.012**	-1.882, -0.239
Age	-0.021	0.001	*<0.001**	-0.023, -0.019
Height	0.025	0.003	*<0.001**	0.020, 0.030
Weight	0.006	0.001	*<0.001**	0.003, 0.009
Use of BMF	-0.057	0.034	*0.093*	-0.123, 0.009
Having pet dog/cat	0.066	0.031	*0.035**	0.005, 0.128
Passive smoking	-0.064	0.037	*0.082*	-0.0135, 008
$FEF_{25\%-75\%}$				
Constant	-1.733	0.808	*0.032**	-3.321, -0.145
Age	-0.021	0.002	*<0.001**	-0.025, -0.016
Height	0.030	0.005	*<0.001**	0.019, 0.040
Weight	0.008	0.003	*0.002**	0.003, 0.013
Use of BMF	-0.073	0.065	*0.261*	-0.201, 0.055
Having pet dog/cat	0.053	0.061	*0.380*	-0.066, 0.172
Passive smoking	-0.061	0.071	*0.386*	-0.200, 0.077

W_{BMF}: Women using biomass fuel as primary cooking fuel, W_{LPG}: Women using liquid petroleum gas as primary cooking fuel, Those with kyphosis (n=5) were excluded, Obstructive airway disease - FEV_1 ≤80%, FVC normal or decreased, Absolute FEV_1/FVC ratio <0.7, Restrictive airway disease - FVC ≤80%, FEV_1 decreased or normal, Absolute FEV_1/FVC ratio >0.7, Small airway disease - $FEF_{25-75\%}$ ≤65%, *p-values* obtained from multiple linear regression. **p<0.05* is significant.

Prevalence of pulmonary dysfunctions

The prevalence of pulmonary dysfunctions as assessed by obstructive airway disease, restrictive airway disease and small airway disease of women primary cooks are depicted in Table 5. Accordingly, a significantly higher prevalence of obstructive airway disease, restrictive airway disease was observed among W_{BMF} than W_{LPG} ($\chi 2$ [3] = 14.2, *p<0.05*).

Table 5: Prevalence of obstructive airway disease, restrictive airway disease and small airway disease among women primary cooks

	Women primary cooks n (%) n=561	W_{BMF} n (%) n=360	W_{LPG} n (%) n=201
Normal	418 (100)	260 (62.2)	158 (37.8)
Obstructive airway disease[#]	22 (100)	20 (90.9)	2 (9.1)

Restrictive airway disease[#]	78 (100)	58 (74.4)	20 (25.6)
Small airway disease	43 (100)	22 (51.2)	21 (48.8)

W_{BMF}: Women using biomass fuel as primary cooking fuel, W_{LPG}: Women using liquid petroleum gas as primary cooking fuel, Those with kyphosis (n=5) were excluded, Obstructive airway disease - FEV_1 ≤80%, FVC normal or decreased, Absolute FEV_1/FVC ratio <0.7, Restrictive airway disease - FVC ≤80%, FEV_1 decreased or normal, Absolute FEV_1/FVC ratio >0.7, Small airway disease - $FEF_{25-75\%}$ ≤65%, [#]Prevalance was significantly different between W_{BMF} and W_{LPG} at p<0.05, Chi-square test with Bonferroni post hoc test

DISCUSSION

The present study investigated the pulmonary functions of women primary cooks and its association to the cooking fuel used. To the best our knowledge, this is the first study to report the pulmonary functions of women primary cooks in Sri Lanka in detail using quantified tests of pulmonary functions. The exposure to cooking fuel smoke depends on cooking practices and the featenvironment,king environment and these factors are driven by traditional socio-cultural influences. Sri Lanka has traditional cooking practices that are passed from generation to generation and have distinctive features in the kitchens. Thus, the study population of the present study is unique. The present study showed that majority of women primary cooks in rural areas still rely on wood and plant material as the main cooking fuel but there is a decrease in the use of biomass fuel over the years from 80% in 2012 to 64% in the present study (28,29). However, as stated earlier the ongoing economic crisis in Sri Lanka may have affected this declining trend but current data is not available. The present study also considered a range of exposures to other air pollutants that may confound the main outcomes of the study (21). Except for a few, the majority of the study participants in both groups have never smoked, and many of these contributors were not significantly different among W_{BMF} and W_{LPG}. Thus, the cofounding effects produced by the exposure to other air pollutants on the study outcomes of the two groups were minimal in this study.

According to the present study, W_{BMF} were significantly older than W_{LPG} which is observed in the studies from other countries as well (11,30–33). Thus, it is consistent that the use of biomass as cooking fuel has been gradually phased out and is being replaced with liquid petroleum gas in the younger generation. The W_{BMF} spent more time on cooking than W_{LPG} because biomass fuel cookstoves have poor thermal efficiency, which is as low as 5-20%, and had poor heat transferring capability requiring more time to cook (34,35). Therefore, the women using biomass fuel are compelled to spend more time for cooking inhaling air contaminated with biomass fuel smoke. Biomass fuel smoke exposure index has been described in the literature as a simple mean of assessing the exposure to biomass fuel smoke, based on the time spent on cooking (36). A study in India reported that the minimum threshold of the biomass fuel smoke exposure index to develop chronic bronchitis was 60 hours-years (36). However, the mean biomass fuel smoke exposure index of this study population was nearly three times greater than this value, which is a cause of concern.

In the present study, the mean FEV_1, mean FVC, and mean $FEF_{25-75\%}$ of W_{BMF} were significantly lower than that of W_{LPG} (*$p<0.05$*) and similar findings were reported in the studies elsewhere (37–42). The "Burden of Obstructive Lung Disease (BOLD)" study reported a higher mean FEV_1 (1.9 L±0.5SD) and a higher mean FVC (2.3 L±0.6SD) for solid fuel smoke-exposed population in Sri Lanka, compared to the present study (43). This discrepancy could be due to the differences in the two study populations, with 46.3% of males in the BOLD study, whereas the present study was conducted only among females. As shown in the present study, age and height are considered as the most explanatory variables of these spirometry parameters (44–46). With aging, there are age-

related anatomical, physiological, and immunological changes occur in the respiratory system (47). Further, with the increasing age, there is a proportionate increase in the years of cooking and thus, the years of exposure for biomass fuel smoke. Thus, two variables had a multicollinearity relationship statistically but in clinical context all these effects of aging and increased exposure to biomass fuel smoke contribute to the pulmonary dysfunction observed among W_{BMF} in this population.

The multiple linear regression model provided promising evidence for the decline in pulmonary functions of W_{BMF} which is similar studies (37,48). Accordingly, W_{BMF} had a reduction of FEV_1 of 0.066 L, FVC of 0.057 L and $FEF_{25\%-75\%}$ of 0.073 L compared to a W_{LPG} when controlled for the above variables but the result was significant only for FEV_1 ($p < 0.05$). The FEV_1 is considered as a sensitive measure of rapid decline in pulmonary functions in smoking populations (49). An accelerated decline in FEV_1 was seen in COPD (50). Thus, the decline in FEV_1 was significant over FVC and $FEF_{25\%-75\%}$. Spirometry is the most commonly used lung function test and is an essential tool for the diagnosis of respiratory dysfunction. Thus, it is an invaluable tool that can be used to screen respiratory functions of venerable populations in the community setting which needs to be popularised in Sri Lankan public health system. Further, obstructive airway disease and restrictive airway disease were significantly higher among W_{BMF} than W_{LPG} ($p<0.05$). The prevalence of airflow limitation, reported in the BOLD study for Sri Lanka, was much higher than what is reported in the present study (7.8% vs. 5.6% respectively) (43). This could be due to that fact that the BOLD study was conducted in a wider population and contributed by both COPD of tobacco smoking and exposure to biomass fuel smoke while the present study was conducted in a focused women population with lower prevalence of tobacco smoking.

There are strong similar evidence is available, stressing the association of exposure to biomass fuel smoke and COPD, but the risk reported in different studies vary (39,40,42,51–53). A higher risk of obstructive airway disease than what is reported in the present study was shown among rural Indian women (39). Despite the older age and increased duration of cooking, the W_{BMF} in the present study showed a lower prevalence of obstructive airway disease compared to that of other countries (54–57). In comparison to other countries, none of these women used animal dung which is considered to produce most polluted emissions (58–60). None of them engaged in cooking in un-partitioned areas whereas in other countries living and sleeping areas were not separated from the cooking area (61–64). Most of them used improved thermally efficient cookstoves placed at a platform at the waist height which could contribute to lower level of exposure (65,66).These women chop the wood and dry in the sun which favours complete combustion, resulting in a less polluted emission (51,67). Thus, these women may have relatively low level of exposure to biomass fuel smoke due to the features in cooking areas and cooking practices that may contribute to the lower prevalence of obstructive airway disease observed in this population. An air quality assessment study preferably, using particulate matter analysis is required to provide quantitative evidence on the level of exposure to biomass fuel smoke.

Limitations

The study design was a descriptive cross-sectional study. The women primary cooks were selected using representative sampling from the study population, based on the inclusion and exclusion criteria. Thus, the opportunity to select W_{BMF} and W_{LPG} with matching age, height, weight, and other air pollutant exposures was limited. However, these confounding effects were addressed at the statistical analysis and the conclusions were derived after correcting for confounding effects.

As the study was conducted in a community set-up away from the respiratory research laboratory, the post-bronchodilator response to spirometry was not assessed. Therefore, the reversibility of the airflow obstruction could not be detected at the field level. Obstructive airway disease was defined based on the "fixed cut-off value of $FEV_1/FVC < 0.07$ of GOLD criteria". This may underestimate the airflow limitation in younger individuals and overestimate the airflow limitation in older individuals (68). Because of the cross-sectional

nature of the study, data collection was done at one point of time. The women who were categorized as W_{LPG} at the time of data collection could have been exposed to biomass fuel smoke previously. The fetal and child exposure to biomass fuel smoke affect the development and maturation of the lungs causing impaired pulmonary functions in the adult life (69,70). Such effects were not considered in the study. Our study, at this stage failed to quantify the concentrations of the air pollutants in the kitchens in both groups. Therefore, the assessment of exposure to biomass fuel smoke is based on the duration rather than the concentration of air pollutants.

CONCLUSION

The W_{BMF} had significantly lower pulmonary functions when compared to W_{LPG}. Age, height, and weight were significant predictors of FVC, FEV_1 and $FEF_{25\%-75\%}$. The use of biomass fuel was significantly associated with a decline in pulmonary functions (as indicated by decline in FEV_1) of women. The prevalence of obstructive airway disease was significantly higher in W_{BMF} than W_{LPG}. The switching to cleaner cooking fuel is recommended to safeguard the pulmonary functions of women. However, this might not be possible due to the poor socioeconomic status observed among W_{BMF} thus modification of the kitchen environment and improve cooking practices will help in reducing the decline in pulmonary functions of W_{BMF}.

Author declaration

Author contributions

GUJ planned the study, conducted the data collection, performed statistical analysis, and drafted the manuscript. SWW and SEG supervised the proposal development, data collection, statistical analysis and edited the manuscript. All authors read and approved the final draft.

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Conflicts of Interests

Authors have no conflicts of interests to declare.

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Ethical Approval

Ethical approval for the study was obtained from the Ethics Review Committee, Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka (ERC No:56/17). Administrative clearance was obtained from the Regional Director of Health, Colombo, Sri Lanka. Informed written consent was obtained from all participants.

Data Availability

Data will be available upon request from the corresponding author.

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Sri Lanka Journal of Physiology **SLJPHYSIOL**

Perspective

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Heart Rate Variability: How it evolved

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Heart rate variability refers to the beat-to-beat variation of the R-R interval of the electrocardiogram. The variability of the heart rate from one beat to the next is a feature of a healthy cardiovascular system. It is well known that the automatic rhythmicity of the heart set by the pacemaker tissue. However, it is also known that this is continuously altered by the autonomic nervous system and many other factors. The beat-to-beat variation of the heart rate or heart rate variability (HRV) is a very popular concept studied by scientists in the last few decades.

In this write up I intend to take you through some of the major historic landmarks in relation to development of HRV and its use in clinical practice. In ancient times man noticed that the heart beat varied, increasing, for example, during physical exertion or sexual arousal. Several Greco-Roman physicians like Herophilos, Archigenes and Galen of Pergamon described heart rate (measured by pulse) the written evidence is fragmentary [1, 2, 3]. Undoubtedly the most influential physician of his times, Claudius Galen (or Galen of Pergamon) wrote at least 18 books on the pulse including many that described using pulse for the diagnosis and predicting the prognosis of disease. His teaching on pulse dominated medical practice for almost sixteen centuries through the Middles Ages and beyond the Renaissance.

In the early eighteenth century, John Floyer (1649–1734), an English physician, invented what he called the "The Physician Pulse Watch," a portable clock which enabled more accurate measurement of time, and therefore more quantitative evaluations of heart rate [4]. He tabulated variation of pulse and respiration under various conditions.

In 1733, the Rev. Stephen Hales (1677–1761) was the first to report that the beat-to-beat interval varied during the respiratory cycle [5]. In 1847, Carl Ludwig using his smoked drum kymograph demonstrated quickening of arterial pressure waves during inspiration and slowing during expiration, the first description of what is known as respiratory sinus arrhythmia [6].

It was in the late nineteenth and early twentieth century that Willem Einthoven (1860–1927), using galvanometers to measure accurately changes in electrical currents, produced the first continuous recordings of the electrical activity of the heart [7]. There dawned the era of recording of electrocardiogram of the heart [7, 8, 9]. Development and standardization of the electrocardiogram took place over the decades that followed. It became possible to evaluate many aspects of ECG could be studied with ease and accuracy.

Another important landmark was the development of the facility to obtain ambulatory electrocardiograms in the early 1960s, which enabled recording over a longer period of time like 24 hours. Norman "Jeff" Holter [10] used a small portable recorder for this and using it further highlighted the beat-to-beat variation in the heart interval. With the development of modern digital signal processing techniques [11], it became possible to quantify and to analyze HRV as a cardiovascular parameter.

The clinical relevance of HRV was first appreciated when Hon and Lee [12] noted that HRV predicted fetal distress. Thus began the golden era of HRV research. Simple Time-domain analysis of HRV developed in the 1960s and continuously being used. In the early 1970s several groups applied power spectral analysis to investigate the physiological basis for the individual frequency components that compose the periodic variations in heart rate [13-21]. Since these pioneering studies the field has rapidly expanded. Both time and frequency and time domain techniques have been used to quantify HRV.

Towards the latter part of the 1970s Ewings and others developed a number of simple bedside tests of R-R interval differences to detect autonomic neuropathy in diabetics [22-24].

Recently, techniques derived from the chaos theory has been used to evaluate the non-linear dynamic characteristics of HRV [25-29].

Measurement standards of HRV are established today by several bodies. The European Society of Cardiology and the North America Society of Pacing and Electrophysiology published guidelines to standardize terminology and methodology of HRV measurement (1996) [30]. Later in 2015, A position paper was published by the European Society of Cardiology and the European Heart Rhythm Association to include the newer non-linear techniques adding to the already existing guidelines of the Task force [31].

The physiological basis underlying HRV, although beyond the purview of this brief report, has been under intensive study and some aspects of it still remains unresolved. As of today, it is believed that the rhythmic changes in the heart rate at any given time reflect the complex interactions between parasympathetic nerve fibers, sympathetic nerve fibers, mechanical, and other factors on the pacemaker cells usually located in the sinoatrial node.

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Review

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Emerging insights on psychiatric pathophysiology

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ABSTRACT

This review describes recent thinking on pathophysiology in major psychiatric syndromes; both neurodevelopmental and neurodegenerative. Interventions via activation or suppression of microglial functioning, use of psychedelics, and potentially, epigenetic manipulation of the genome are discussed. There is also increasing interest in relation to mood disorders on activity of the gut biome, which appears to 'educate' the gut immune compartment to self-regulate. Initial trials using faecal transplantation have commenced to assist mood disorders. Regular exercise of different types appears to reduce systemic and cortical inflammation, improve neurogenesis, and maintain physical health in people with severe mental illness who typically die of cardiometabolic disease or cancers 15 – 20 years prematurely.

INTRODUCTION

Academic psychiatry is currently undergoing a major rethink due to invalidation of 3 key hypotheses involving 'chemical imbalances' being of aetiological significance. Specifically, it is apparent that major mood disorders as a group are not associated with deficits of Serotonin production or distribution[1]; the basis of the 'monoamine hypotheses of mood disorders. Furthermore, the Dopamine hypothesis of schizophrenia - symptoms being caused by excess Dopamine production - appears to be overly simplistic, as inactivity of the pre-frontal cortex appear to be the precursor for Dopamine being diverted to the striatum leading to positive symptoms[2].

Finally, Alzheimer's dementia does not appear to be due to a specific deficit of Acetyl Choline (the Cholinergic hypothesis) or excess production of the oligomer amyloid beta (the Amyloid hypothesis); the likely explanation being inadequate clearance of misfolded proteins in general.[3] with secondary effects on various neurochemicals in addition to Acetyl Choline[4]. This is not surprising as these hypotheses have not led to clearly effective treatments.

RESEARCH DEVELOPMENTS

Recent epigenetic studies show DNA Methylation mediating effects of childhood trauma, for example making glucocorticoid receptors more sensitive to stress in adult life[5], these changes potentially being passed on to subsequent progeny. There have also been significant improvements in volumetric measurements in structural brain imaging, allowing serial scanning to detect changes. Techniques such as functional Magnetic Resonance Imaging (fMRI) are used to detect activity of specific brain regions when subjects are engaged in specific thought experiences and during cognitive testing[6].

Radioactive ligands have shown deficits in Potassium and NMDA channels associated with a condition called 'limbic encephalitis'; a mixture of psychotic, mood and cognitive symptoms presenting in around 5% of those diagnosed with psychosis.[7] Furthermore, transmembrane Sodium / Water pump malfunction has been linked to bipolar disorders, with Lithium being able to remedy these pumps to prevent recurrence[8]. Consequently, bipolar disorder is seen as a multi-tissue (renal, thyroid, and dermal) condition. On obsessive compulsive and other habit disorders, a 'reverberating circuit'[9] involving the medial prefrontal areas, thalamus, hippocampi and anterior cingulate has been identified using Diffusion Tensor Isometry (DTI) imaging tracking movements of water.

Recently, psychedelics such as Psilocybin, MDMA and Ketamine have been found highly effective in treating Post Traumatic stress Disorder (PTSD), mood disorders and compulsions[10]. These treatments can be administered in group settings, with psychotherapy debriefing. There is also growing interest in the gut biome, mainly involving rodent research, suggesting that faecal transplantation (and introduction of specific species like Lactobacillus) could benefit chronic anxiety and depression. The recent pandemic involving SARSCov2, has accelerated interest in the innate immune system; specifically, how microglia (the scavenger cells in the brain) move from its neuroprotective state to a hyperinflammatory state, consequently failing in its phagocytic function of clearing insoluble oligomers such as beta amyloid[11].

SYNAPTIC PRUNING PATTERNS IN NEURODEVELOPMENTAL DISORDERS

Neuronal synaptic pruning allows the brain to 'fine tune' neuronal circuits and maintain neuronal plasticity. Pruning is carried out by microglia which, in its usual neuroprotective state, uses cytoplasmic projections to check surrounding synapses. Local messengers called chemokines (alongside ATP and Glutamate) generate 'find me' and 'eat me' signals to activate microglia in its phagocytic role. Rapidly replicating neurones produce a chemokine called Fractalkine which binds to the microglial receptor CX3CR1 causing microglial activation[12]. Furthermore, astrocytes secrete a cytokine - Transforming Growth Factor beta (TGFβ) - to assist microglia prune synapses via the complement cascade (C1q, C4, C2 and finally C3). Microglia can also be activated (specifically in the pre-frontal cortex) by systemic inflammatory cytokines such as Interleukin 6 (IL6) in the presence of chronic stress. There is genome directed blockade of CX3CR1 to reduce microglial activation, promoting neurogenesis and synaptic growth[13]. Overall, a healthy brain maintains a balance between synaptic pruning and synaptic proliferation.

There are 3 main synaptic pruning seasons. Firstly, from birth up to the age of 2 years, there is a whole cortex spell of cortex wide pruning to assist the formation of sensory-motor circuitry. Thereafter a further spell of pruning is observed between the ages of 11 and 15, with degree of variability between the sexes to integrate motor and emotional responses. Both these pruning spells are genetically directed in terms of onset and duration. A further spell of more selective pruning involving the pre-frontal cortex occurs in early adulthood (18 – 25 years), to develop executive and team-based activity. This spell of pruning appears to be mainly directed by life events, for example leaving home or commencing work.

It has been recently hypothesised[14] that variations to the typical patterns of pruning are associated with neurodevelopmental disorders. Markedly reduced generalised pruning appear to be linked with autism. A related condition attention deficit hyperactivity disorder (ADHD) appears to be linked to reduced extent of pruning, with full pruning achieved in adulthood. Schizophrenia, bipolar and

(the hybrid) schizoaffective disorder appear to be associated with excessive pruning during the usual seasons; much more generalised in the former particularly affecting the pre-frontal areas, with more localised deep pruning occurring in bipolar / schizoaffective conditions, for example bilateral anterior and subgenual cingulate cortices, areas related to mood awareness. Threatening life events also appear to 'reignite' pruning in these conditions out with the typical seasons.

Evidence for this hypothesis involves longitudinal MRI scanning of adolescents presenting with schizophrenia compared to siblings and healthy controls[15]. Over a 5-year period, results showed a fourfold excess of grey matter loss in the schizophrenic adolescents compared to controls, with their siblings showing a similar loss which ameliorated thereafter. Furthermore, a PET study has shown evidence of excess microglial activation in people with schizophrenia compared to control subjects[16]. Immunological studies in schizophrenic patients have shown alterations in the complement cascade with excess activity of C1, C3 and C4 compared to controls with less activity of C2[17].

This hypothesis is also consistent with the variation in recovery from first onset psychoses, as genes directing neurogenesis such as Brain Derived Neurotrophic Factor (BDNF) could be demethylated and allowed to function maximally during recovery, reversing some of the excess pruning produced during the acute illness. Alternatively, patients with limited neurogenesis will maintain cognitive and volitional deficits leading to 'chronic' schizophrenia. As for ADHD, imaging suggests levels of synaptic pruning to be less than that observed in schizophrenia, with evidence of slower maturation of the frontal / pre-frontal areas[18]. In autism, DTI imaging has shown limited long track connections between the different brain lobes alongside extensive local connections (resulting in heavier brains due to excess myelination). Reduced overall microglial activation has also been observed - except for the dorsolateral prefrontal cortex.[19]

Treatments being considered to reduce excess pruning includes Minocycline, which reduces microglial activation; currently used in psychosis and resistant depression as augmentation[20]. Alternatively, increasing neurogenesis to recover damage caused by excess pruning could involve increasing the expression of BDNF via epigenetic means or via exercise, sleep hygiene and occupational activity. Furthermore, immunological interventions increasing T regulator cells has prophylactic neuroprotective potential[21]. These cells, largely expressed in the gut lamina propria in response to gut microbiota, can move to the brain parenchyma via cranial venous sinuses and mitigate excess inflammatory activity.

RECENT INSIGHTS ON MOOD DISORDERS

Of late, there have been attempts to disentangle major depression ('melancholia') from dysthymia (dissatisfaction / boredom) often the result of excessive stimulation of the brain reward system[22]. Dysthymia does not necessarily respond to antidepressants. Treatment involves a 'reset' of daily activity including finding a sustainable (perhaps more altruistic) purpose in life. As described before, a 'reverberating circuit' can form due to compulsive behaviours. 'Deep Brain Stimulation' - placing an electrode in the cingulate / prefrontal sector of this circuit has been used successfully, with the patient being able to control the intensity of the impulse depending on symptom severity.

Major Depression is linked with inflammatory microglial activity in the pre-frontal cortex, secondary to excess cortisol production due to threatening life events or secondary to a systemic inflammation caused by physical disease including autoimmune conditions. A variety of non-steroidal anti-inflammatory agents (NSAID's) have been used to augment standard antidepressants in 'difficult to treat' depression[23]. Serotonin reuptake inhibitors have anti-inflammatory properties, as do atypical antipsychotics, which might explain their antidepressant effect in some individuals. Similarly, psychedelic agents used to treat resistant depression have anti-inflammatory properties alongside the ability to stimulate Serotonin ($5HT_2$) receptors in the cortex.

Interest in gut microbiota dysfunction in relation to mood disorders commenced following observations that a single course of antibiotics increased the risk of mood disorders[24], although the direction of causality remains unclear. Similar

concerns have been expressed about long term exposure to dietary Glyphosate, which disrupt tight junctions between endothelial cells, resulting in endotoxins and harmful microbials (E Coli, Clostridia) to leak into the lamina propria resulting in inflammatory T 17 cells being produced from naïve T cells, which then migrate to brain parenchyma causing microglial inflammation and release of cytokines such as IL17, IL6, TGF alpha. Conversely, a healthy gut microbiota stimulates the development of T regulator cells which controls inflammation both in the body and brain. These ideas have led to experimentation with probiotic species (like Lactobacillus) and faecal transplantation (via ingested capsules from a suitable doner) to reverse mood disorders[25]. Faecal transplantation has a good safety record, having been used to treat C. Difficile infections.

There are 2 other methods whereby the gut microbiome communicates with the brain. The vagus nerve traverses the gut, the heart, and the brain[26]. There is bidirectional transfer of impulses, and a healthy gut and microbiota can produce vagal impulses which stabilises the neural network of the heart as well as circuits in the limbic system. Similarly anti-anxiety cognitions and behaviours - for example utilising mindfulness and yoga - will assist the heart and the gut to be more relaxed. Electrical stimulation of the Vagus Nerve has been used for the adjunctive treatment of atypical depression. Furthermore, excess activation of the Hypothalamic-Pituitary-Adrenal (HPA) axis – for example involving unremitting stress or melancholia - can cause effects both on the gut epithelium (loss of integrity, inflammation of the immune compartment) and the gut microbiota (reduced flora)[27].

Epidemiological findings link PTSD and other anxiety disorders with childhood maltreatment including coercive control and emotional neglect[28]. Also, chemokine patterns (studied in US marines pre and post deployment) suggest different signatures separating PTSD victims from those with general stress and those who are asymptomatic[29]. Increasingly psychedelics such as Psylocibin are being used to treat intractable PTSD, although the rationale for benefits in PTSD is yet to be fully elucidated, as subjects often describe a 'life changing' experience following a single administration. PTSD is associated with a high suicide rate (even compared to major depression); currently Ketamine by infusion is being used for its rapid onset anti suicidality property[30].

INSIGHTS ON DEMENTIAS POST COVID19

The main issue in avoiding dementia is how to clear misfolded proteins (oligomers) from the brain parenchyma[31]. Each type of dementia is characterised by presence of different oligomers; Amyloid Beta (Aβ) in Alzheimer's Disease, Alpha Synuclein in Parkinson's dementia and a combination of Tau and DP-43 in Frontotemporal dementia. Vascular ischaemia appears to lower the threshold for clinical manifestations to appear during life, alongside alcohol / lead poisoning, repetitive brain trauma, and encephalitis with Herpes and Cytomegaloviruses (both found in post-mortem brains). Around 25 years ago, a vaccine containing Aβ antigens was administered to subjects with Alzheimer's disease, which showed clearance of amyloid from brain parenchyma, but deposition in brain glymphatics, at times causing meningoencephalitis[32]. Consequently, vaccine development has been slower, but recent improvements in mRNA vaccine technology has prompted a combined Aβ and Tau antigenic vaccine currently undergoing clinical trials.

The other option is utilising the non-specific effects of repurposed vaccines in improving performance of the innate immune system, for example utilising BCG boosters. It has been suggested that these can improve the phagocytic function of microglia in clearing oligomers[33]. Indeed, there is evidence that BCG boosters can reduce the incidence of conversion to clinical Alzheimer's disease; a serendipitous finding in a trial to treat bladder cancer[34]. Currently a trial of the BCG booster in mild Alzheimer's patients is under way at Massachusetts General Hospital. In the meantime, varieties of passive immunity via antibody mixtures are being used with variable benefit.

There is concern about neuropsychiatric sequelae of 'long Covid' (symptoms lasting over 6 months after the initial infection). A recent retrospective 2 year follow up study of over 1.5 million subjects diagnosed with Covid19 showed persisting features (significantly more than that reported by the non-infected); new psychoses, seizures, and

dementias[35]. There was also excess of subjective 'brain fog'. In contrast, anxiety and depression temporarily increased following infection but resolved spontaneously; typically, within 6 months. Neuroimaging studies have shown whole brain volume loss in subjects infected with SARSCov2[36], with evidence of microglial overactivity. Although there is no evidence that viral particles are evident in brain parenchyma, systemic inflammatory effects including reduced T regulator cells and inflammatory cytotoxins are observed. Potentially the threshold for clinical manifestations of dementias in a larger population of older adults could be lowered.

On detecting early Alzheimer's disease (around 60% of dementias in the over 65 population), there are genetic tests such as allelic subtyping of ApoE, and measurement of the ratio of Aβ 42/40 in cerebrospinal fluid to select those requiring further investigation through imaging. Structural brain imaging also detects the extent of small vessel ischaemia ('white matter burden'), suggesting prognosis and life span. Oligomers are also present in serum; better detection techniques will assist non-invasive screening of the pre-symptomatic. There is evidence that reversing deafness, treating hypertension, glucose intolerance, fatty liver and maintaining cognitive function delays clinical manifestations of Alzheimer's and related dementias[37].

MITIGATING COMORBID PHYSICAL DISEASE AND ASSOCIATED RISKS

Around 75% of patients with severe mental illness (SMI) and dementias die of 'natural causes' (heart and stroke disease, non-alcoholic fatty liver, venous thrombo-embolism, and cancers)[38]. Considering the difficulty in significantly reducing suicide and homicide rates through psychiatric interventions, it does make sense for psychiatrists to prioritise physical healthcare in reducing overall mortality and potentially increasing lifespan. Natural causes also include consequences of atypical antipsychotic use long term, with Insulin resistance due to GLUT transporter blockade[39]. Because of limited Primary Care follow up of patients with SMI and Dementia for a variety of reasons, it seems incumbent on mental health services to monitor physical health of patients under their care. Consequently, most mental health services are setting up physical health hubs. These hubs can also initiate treatments, for example, Lithium, Clozapine, depot antipsychotics and Ketamine infusions.

There is overwhelming evidence that exercise has beneficial effects on the physical health, alongside mental health benefits. Aerobic exercise including walking and racquet sports release Nitric Oxide (NO) from the endothelium[40]. NO is the body's natural hypotensive agent lubricates the endothelial surface reducing the risk of clot formation. The other area of concern is sarcopenia amongst psychiatric patients due to inactivity; potentially accompanied by fat infiltration between and within muscle groups (linked with dyslipidaemia due to fatty liver). Anaesthetists report that people admitted to Critical Care (for example with sepsis or in Phase II Covid19) have worse outcomes on ventilation when sarcopaenic obesity is present[41]. In elderly patients, sarcopenia can lead to gait instability, falls and fractures, precipitating death. Awareness of sarcopenia and sarcopaenic obesity is limited among psychiatrists; post graduate educators are starting to increase awareness. Resistance exercise is best for preventing sarcopenia[42], alongside increasing oral protein. The other area needing more awareness is the risk of recurrent aspiration - for example in people with dementia treated with atypical antipsychotics to manage 'challenging' behaviour. This leads to community acquired pneumonia, another cause of premature death.

CONCLUDING REMARKS

This article describes a plethora of new insights and potential treatments to manage the physical and mental health manifestations of psychiatric illness. However, a degree of caution needs to be exercised as research backing these are often based on single studies without replication. As suggested by Karl Popper, a single well-designed study with a negative result can nullify multiple positive studies[43] as has occurred on treatments for SARSCov2.

The importance of educating and increasing awareness of psychiatric pathophysiology and

treatments has been touched on. It is equally important that nursing staff and lay people (for example carers of those with psychiatric illness) should also be included in education, to reduce stigma and maintain concordance on treatments with evidence of effectiveness. Education on sleep hygiene, foods providing essential nutrients and appropriate exercise is also beneficial for psychiatric patients with a view of mitigating reduced life expectancy.

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Regulation of Energy Homeostasis by Brown Adipose Tissue - "A fat lot of good?"

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Homeostasis is the maintenance of the internal environment within narrow limits. Physiological variables such as blood pressure, and blood glucose level are maintained within narrow limits. Similarly, body weight is also maintained within narrow limits by homeostatic mechanisms dealing with energy balance. Unfortunately, most medical curricula do not include the physiological regulation of energy balance, despite the current epidemic of overweight and obesity.

The proportion of obese men has more than tripled and the proportion of obese women has more than doubled since 1975. According to the World Health Organization, at least 3.4 million people die each year as a result of being overweight or obese. When the disease burden is considered, 44% of Type-2 diabetes, 23% of ischemic heart disease and between 7 - 41% of certain cancers are attributable to obesity.

According to a report by McKinsey Global Institute, obesity is one of the top three social burdens generated by humans. The global economic impact of obesity amounts to roughly 2 trillion dollars annually, nearly equivalent to the global impact of smoking or of armed violence, war, and terrorism. Once associated with high-income countries, obesity is now also prevalent in low- and middle-income countries as well. In the Colombo Urban Swas, led by Dr. Noel Somasundaram, we found that a staggering 65% of adults in a representative area of urban Colombo were overweight or obese. Earlier, we have shown that in a sample of close to 3000 adult males from the Central Province, the prevalence of overweight and obesity was 44%.

Obesity is a complex chronic disease which gives rise to numerous complications. For example, in this recent paper, we showed that non-alcoholic fatty liver disease is strongly associated with obesity in adult Sri Lankan women. We also know that obesity increases the risk of pre-mature death, type-2 diabetes, cardiovascular diseases (CVD) and cancer.

When the body has surplus energy from excessive food intake, it is converted to fat and stored in a specialized tissue called the adipose tissue. Adipose tissue can be classified into white, or brown based on its structure and function. White adipose tissue, which stores fat during states of excess energy availability, can influence whole body homeostasis via the secretion of adipokines and inflammatory mediators. Now we know that adipocyte death and adipose tissue hypoxia are two triggers for inflammatory adipokine secretion in obesity, which contributes to the pathogenesis of insulin resistance. For example, in this recent paper, we showed that adipose tissue production

of the adipokine resistin is linked to dysglycemia in adults Sri Lankan women. Brown adipose tissue, in contrast, is important in energy dissipation via non-shivering thermogenesis, especially in newborns. More recently, another type of adipose tissue was identified in white adipose tissue which was termed brite ("brown in white") or beige adipose tissue. Brite adipocytes can stem from progenitors or transdifferentiate from mature white adipocytes by a process called browning. Therefore, brown and beige adipose tissue have an anti-obesity potential and are deemed beneficial in terms of preventing / reversing obesity-associated complications.

Energy homeostasis is regulated at the level of the hypothalamus. There are several inputs from the intestine and white adipose tissue which provide information about the energy status of the body, which are integrated at the hypothalamus, which in turn sends efferent signals to change food intake and energy expenditure. The efferent pathways to brown adipose tissue, which increase energy expenditure, are important for both weight loss and weight maintenance strategies. Therefore, studying the physiology of brown adipose tissue as well as ways to increase its activity would be of great benefit in the management of obesity and weight maintenance. Brown adipose tissue was traditionally known to be important in heat production in hibernating animals and new-born infants. Cold exposure is the most potent activator of brown fat.

So how does brown adipose fat produce heat? Energy substrates such as fatty acids and glucose eventually enter the TCA cycle in mitochondria producing NADH and FADH2. These enter the electron transport chain, where a proton gradient is established across the inner mitochondrial membrane. Under normal circumstances, flow of these protons through ATP synthase drives ATP synthesis. In brown fat, there is a special protein called uncoupling protein 1 or UCP1 in the inner mitochondrial membrane. Protons can also flow through this protein like through ATP synthase. However, no ATP is produced in this instance and energy is lost as heat instead. Thus, UCP-1 is key to the heat production by brown fat. At the whole animal level, cold exposure leads to sympathetic activation which stimulates beta-3 adrenoceptors in brown fat cells, which in the short-term increases lipolysis to provide substrate for thermogenesis in brown fat. In the long term, chronic beta-3 adrenoceptor activation ultimately leads to increased UCP-1 expression.

What are the physiological activators of brown fat and browning? We already know that cold is a potent activator. There is some evidence that exercise can also promote browning via the mediator irisin. However, the evidence for the latter is still unconvincing. We, in our research group asked the question whether dietary bioactive compounds such as omega-3 fatty acids can activate brown fat or browning. In a previous study, we found that the omega-3 fatty acid EPA, which is found in fish oil, can prevent high-fat diet-induced excessive weight gain in mice. Since there was no difference in energy intake or feed efficiency between the high fat and the high-fat EPA groups, we speculated that the lesser weight gain by EPA is probably due to higher energy expenditure. Unfortunately, we did not have equipment in our lab those days to directly measure oxygen consumption and energy expenditure. More recently, with the renewed interest in brown fat, we looked at the UCP1 expression in brown fat from these same mice. We found that UCP-1 expression was indeed increased by EPA. As expected, there was no UCP-1 expression in the white adipose tissue. Next, we used cultured brown fat cells to study the effects of omega-3 fatty acids in vitro. The mitochondrial density increased with increasing omega-3 concentration illustrating a dose-response effect. We concluded that the omega-3 fatty acid EPA activates brown fat by increasing the mitochondrial density and UCP-1 expression.

Next, we wanted to explore the mechanisms responsible for the EPA effects on UCP-1 expression. Protein expression can be regulated at various levels. The central dogma states that DNA or the 'gene' is transcribed into RNA, which is then translated into the protein and that protein expression can be regulated at the level of gene transcription or post-transcriptionally. Micro RNAs are small non-coding RNAs which can degrade m-RNA and can regulate protein expression post-transcriptionally. We used state-of-the art miRNA profiling and found that omega-3 fatty acids induce several micro RNAs in brown fat.

Some of these micro RNAs are involved in UCP-1 gene expression.

In summary, we and other research groups have shown that omega-3 fatty acids can activate brown fat cells and browning by the following mechanisms: One – omega-3 fatty acids bind to the G protein coupled receptor GPR120 which increases cyclic AMP, which in turn increases UCP-1 expression. Two – omega-3 fatty acids modulate micro-RNA expression which can also increase UCP-1 expression. Three – omega-3 fatty acids increase the beta-3 adrenoceptor density in the brown fat cells, which in turn leads to activation of brown fat cells.

It is known that brown fat increases energy expenditure and hence has an anti-obesity effect. Next, we identified that UCP1 is critical for brown fat function. Therefore, it is logical to hypothesize that if UCP1 is absent, there will be a higher predisposition for obesity. So how do we test this hypothesis? As discussed earlier, gene transcription and translation lead to protein expression. Thus, if we delete the gene, there will be no protein. The UCP-1 knockout was performed by Leslie Kozak's lab in Pennington, and they tested the hypothesis that "UCP1 knockout mice will be more prone to high-fat diet-induced obesity". To their surprise, they found the opposite to be true, which is that UCP1 knockout mice were in fact, resistant to high-fat diet-induced obesity. Why is it that in research, our logical hypotheses get rejected most of the time? To quote Douglas Green, chair of immunology at St. Jude children's hospital in Memphis, "Life is not logical, because living things are not designed". However, we should be able to explain our observations scientifically. Laboratory mice are generally housed at a temperature around 23^0C, while their thermoneutral temperature is closer to 30^0C. In the presence of this cold stress, since brown fat is not working, the body resorts to less energy efficient systems for thermoregulation, leading to increased energy expenditure and hence the resistance to obesity. To test this explanation, another group subsequently conducted the same experiment at thermoneutrality and observed that now, the UCP1 knockouts gained more weight compared to their wild type counterparts. In this figure, you can see that the UCP1 knockout mice represented in the upper line in both panels, gained more weight when fed either a control diet or a high fat diet.

Looking at the data from these two studies and from our previous work, we hypothesized that Omega-3 fatty acids protect against obesity in a UCP-1 dependent manner, and therefore, these fatty acids will not be protective against obesity in UCP-1 KO mice. To test this hypothesis, we fed wild type and UCP-1 knockout mice a high fat diet or a high-fat diet supplemented with EPA. The UCP1 knockouts had larger vacuoles in brown fat, making them look more like white adipose tissue. The UCP-1 knockouts also had higher triglyceride content showing that brown fat becomes more white-like in the absence of the key protein UCP1. When we looked at the weight gain of these mice, we were surprised to see that EPA was able to prevent excessive weight gain in UCP1 knockout mice again rejecting our hypothesis. This indicates that EPA is able to prevent high-fat diet induced weight gain in an UCP1 independent manner as well. Next, we measured the oxygen consumption of these mice using a metabolic monitoring system. The ucp1 knockouts fed the high-fat diet had significantly lower oxygen consumption than the high-fat fed wild type group. Moreover, EPA supplementation rescued this reduction in oxygen consumption in the knockouts.

From this series of studies, we can state that Omega-3 fatty acids activate UCP-1, brown fat and energy expenditure and that there are UCP-1-independent mechanisms by which omega-3 fatty acids increase energy expenditure as well. So at this point, is there enough evidence to suggest that these findings from basic studies have a translational value?

In an elegant study by van der Lans and colleagues, 17 healthy subjects were cold acclimated by exposure to an environmental temperature of 15–16°C for 6 hours a day for 10 consecutive days. This led to a significant increase in brown fat activation as well as an increase in non-shivering thermogenesis from 10.8% to 17.8%. On the basis of similar other human studies, it is plausible that humans have the potential to increase their whole-body thermogenic capacity to achieve long-term weight loss. It is concluded that brown fat is an important contributor to total energy expenditure and energy homeostasis. Uncoupling protein-1 is important in maintaining normal function of brown fat. Cold exposure and exercise activate brown fat. Finally, emerging evidence suggests that dietary bioactive compounds such as omega-

3 fatty acids may also activate brown adipose tissue and have an anti-obesity potential.

Author declaration
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Rabies revisited: Past, Present and the Way forward

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Part I

Reporting from the Louis Pasteur's laboratory…….

Louis Pasteur was a French chemist and bacteriologist who became the founder of microbiology. At the inception he had concentrated on studies related to bacteria and put forward his germ-theory of diseases. His investigations had enabled him to develop the process of pasteurization and also to develop vaccinations for many germ-borne diseases. During these days, as rabies was also prevalent, he had extended his investigations into this deadly disease. He had inoculated the rabies virus into different animal models maintained in his laboratory. These investigations led Louis Pasteur to develop his rabies vaccine by growing virus in rabbits, and subsequently drying the affected nerve tissue to weaken the virus ***(https://www.wired.com/2011/07/0706pasteur-rabies-vaccine/).***

The report titled Rabies Vaccine saves boy and Pasteur, indicates, that "On July 6, 1885, the vaccine was administered to Joseph Meister, a 9-year-old boy who had been attacked by a rabid dog. The boy survived and avoided contracting rabies, which would have almost certainly proved fatal. Good thing it worked: Pasteur was not a licensed physician and could have been prosecuted had the vaccine failed. The legalities were forgotten, and Pasteur instead became a national hero" (***https://todayinsci.com/P/Pasteur_Louis/Pasteur-Rabies.html).***

As stated in the report, by the end of 1885, several more desperate rabies-exposed people had travelled to Pasteur's laboratory to be vaccinated. During 1886, Pasteur had treated 350 people with his rabies vaccine, of whom only one had developed rabies.

Pathogenesis of rabies

Rabies is one of the oldest and most devastating zoonotic diseases known since 2300 BC. The word rabies originates from the Latin word rabere which means anger and rave.

Rabies is an acute encephalitis which is an acute infection in the central nervous system caused by a rod or bullet-shaped single-stranded RNA virus belonging to the Rhabdoviridae family.

When a rabid animal bites or scratches a person rabies virus is inoculated and gets multiplied at the site of the infection. Subsequently, the virus binds to the nicotinic acetylcholine receptors at the neuromuscular junction and enters the axons of the peripheral nervous system. Upon entering the spinal cord, the virus travels to the brain. Viral

infection of brain neurons leads to their dysfunction causing rabies which is a deadly zoonotic disease.

Global perspective in brief

According to WHO 2018 position paper, rabies is responsible for an estimated 59,000 human deaths and over 3.7 million disability-adjusted life years (DALYs) lost every year at the global level (*https://www.who.int/publications/i/item/who-wer9316).*

As per the 2016 report jointly published by the Food and Agriculture Organization of the United Nations, World Health Organization, World organization for animal health and Global Alliance for Rabies control, countries like Australia, Great Britain, Japan and the United States of America are free of rabies. Most of rabies cases occur in Africa and Asia, with approximately 40% of cases in children aged < 15 years. This deadly Zoonotic disease is endemic to Sri Lanka. Rabies been a major public health problem imposes a heavy economic burden too. The total estimated cost of the activities undertaken for its prevention and control is approximately US dollars 8.6 billion per year at the global level.

Part II

Dog Vaccination

All mammals are susceptible to rabies. The virus transmission happens via two cycles namely the urban and sylvatic cycles. The dog is the principal transmitter of the virus in the urban cycle while wild animals such as foxes, wolves, jackals, bats, raccoons, skunks, or mongooses are involved in the sylvatic cycle. The contribution of the sylvatic cycle in rabies virus transmission is very low compared to dog-mediated rabies and the latter is responsible for up to 99% of human cases in rabies-endemic countries including Sri Lanka.

Therefore, vaccination of dogs against rabies is identified as an important control measure to eliminate rabies. However, as per the recommendation of Global organizations vaccination coverage in dogs should be more than 70% in high-risk countries in order to establish and maintain herd immunity against rabies and thereby break the rabies transmission cycles.

According to the information of Public Health Veterinary Services of Ministry of Health, there is a reduction in the number of human deaths over the last 40 years in the country due to dog anti-rabies vaccination programmes and human post-exposure treatments. The number of human deaths recorded in 2020 is 31. This indicates that zero human rabies deaths could be an achievable target for our country.

According to dog population surveys conducted as part of the PhD study by Dr Ruwini Pimburage, the total dog population is approximately 3 million. Among them, approximately 30% is free roaming. This makes it difficult in achieving WHO-recommended dog vaccination coverage.

Vaccination of dogs leads to the development of immunity against the rabies virus. There are several factors that affect immunity development following vaccination. Among the factors, the potency and efficacy of the vaccine are two important considerations. Potency is the ability to exert the desired effect in dogs following vaccination. Efficacy is a measure of lowering the risk for an identified disease.

The efficacy of vaccination against disease could be determined by the immune response generated. Humoral immunity measures the antibody response which is B lymphocyte-mediated response, and the Cellular immunity or T lymphocyte-mediated immune response is responsible for destroying foreign antigens. Humoral and cellular immunity act against the virus to control the disease if the animal is exposed to the virus.

Also, during the initial or primary immune response which occur in the animal following the first anti-rabies vaccination, memory B and T cells also develop which are useful in boosting an immune response in subsequent anti-rabies vaccinations.

The presence of a proportionately higher percentage of free-roaming dogs in the country is a challenge faced by the authorities in rabies control and elimination. During my clinical practice as a veterinarian, I encountered several occasions of vaccination failures especially in puppies. On the other hand, there was no established mechanism to monitor anti-rabies vaccine efficacy routinely in the country.

This made me initiate immunogenicity studies in dogs following anti-rabies vaccination in the

country in collaboration with the Department of Rabies research and Vaccine quality control of the Medical Research Institute in 1999.

The first project designed was to determine virus-neutralizing antibody titres in dogs or humoral immune response before and after vaccination with the anti-rabies vaccine. This project was funded by the National science foundation and dogs brought from Kotte and its suburbs were recruited for the study.

In this study, adult dogs aged 1 to 5 years and puppies of 3 months old were considered under two groups. Groups were sub-divided considering the previous vaccination history of adult dogs and the vaccination status of the female dogs of puppies recruited. In this study, only domestic dogs were included. Government rabies control programme recommended World Health Organization-approved imported vaccine was injected intramuscularly to animals after collecting day 0 blood samples and post-vaccination blood samples were collected in 1 month, 6 months, and 1 year. Serum samples of blood collected from 52 animals were analyzed for rabies virus-neutralizing antibody titres by Rapid fluorescent Focus Inhibition Test. At the time of commencing this project, the recommendation for the first anti-rabies vaccination for puppies was at the age of 3 months.

The second project was planned as an extension of the first project with the inclusion of apparently healthy stray and domestic dogs. This was conducted by Dr Ruwini Pimburage as the first part of her PhD. National Science Foundation was the funding organization.

Local breed dogs from Kalutara District were recruited for the second project._Considering the age, previous anti-rabies vaccination history and regularity of vaccination of juveniles and adult dogs and the vaccination status of female dogs of puppies, recruited animals were subdivided into eight groups. Serum samples of blood collected on identified time points from 380 animals were analyzed by the Rapid fluorescent Focus Inhibition Test for rabies virus neutralizing antibody titres.

The antibody response of the two projects provided evidence for the efficacy of the vaccine as it has induced an immune response following vaccination. Dogs having a previous vaccination history showed a higher response compared to the dogs without a previous vaccination history.

The lower level of maternal immunity which was below the recommended protective threshold (0.5 IU/mL) in puppies of vaccinated female dogs was evident at the time of the first vaccination.

The pattern of immune response in adult dogs and puppies in the second project was closely similar to the response observed in project 1.

Based on the findings of the two projects our conclusions and recommendations were

Maternal antibodies do not provide adequate protection to puppies until the first anti-rabies vaccination. Immunity development after vaccination seems to be closely similar in both groups of puppies (puppies of vaccinated and unvaccinated female dogs) irrespective of maternal immunization.

The first anti-rabies vaccination for puppies needs to be done before the age of 3 months, as protective antibody titres were absent at that age. Thereafter, a booster vaccine is to be given at a suitable interval, as protective antibody titres were not maintained until 6 months in puppies.

After the recommended two anti-rabies vaccinations to puppies and juveniles in the first year, annual boosters are to be given to all dogs, in order to maintain the antibody titres above the protective level as rabies is endemic to Sri Lanka.

Results of the first project with the recommendations made were submitted to the Ministry of Health as it was the responsible authority that organized the government dog vaccination programme.

The two projects completed, measured actual antibody titres following anti-rabies vaccination in dogs. These findings as the only research-based evidence available in the country were considered to revise the dog anti-rabies vaccination protocol in the country in 2013.

Upon completion of the second project, based on the recommendation of the National Science Foundation, the third project titled Pattern of immunogenicity in domestic puppies and juvenile dogs following primary and subsequent booster vaccination against rabies in the Dehiwela

Municipality area was planned, to look for the effectiveness of the revised dog vaccination schedule. Dogs were recruited, and samples were collected until 1 year following vaccination. The sample analysis is yet to be completed.

Part III

The way forward

The information I mentioned in part II is related to humoral immunity measurement to determine the efficacy of vaccination. There is no established method in the country to determine cellular or cell-mediated immunity following anti-rabies vaccination. There are about seven different brands of Anti-rabies vaccines meant for animals marketed in the country as single-dose and multidose injection vials. Registration of the vaccines is based on information dossiers of these imported vaccines submitted to regulatory authorities by the local agent for the vaccine. The potency of these vaccines has not been tested in the country.

Therefore, a new project was designed to compare the immunogenicity and potency of these different brands of animal anti-rabies vaccines. In this type of study, experimental conditions need to be standardized to minimize variation as much as possible. This new project includes an experimental study involving laboratory-bred rabbits. Thereafter, 3 vaccines of the highest immunogenic capacity and good potency will be selected for the planned field study involving dogs. This is a multicenter collaborative study that includes the Animal Centre of Medical Research Institute, Colombo Municipal Council, Public Health Veterinary Services of Ministry of Health and the international collaborating laboratory. Samples are analyzed by fluorescent antibody virus neutralization test at the WHO/OIE Reference laboratory for rabies and wildlife in France. Once completed this will be the first project at global level where rabbit is used for immunogenicity studies following anti-rabies vaccination.

At the same time, for this project, a semi-quantitative assay for the detection of antibodies that is to check whether antibody titre is at the protective level or not was established in the Department of Physiology, Faculty of Medicine, University of Colombo.

Currently, the method used for anti-rabies vaccine potency testing is the National Institute of Health, mouse inoculation test where quite a high number of mice needs to be sacrificed. During this procedure, mice may develop rabies if the potency of the anti-rabies vaccine tested is not up to the required level. Our attempt is to see the possibility of using immune responses as a substitute for mouse inoculation test.

The attempts that were taken may be useful to achieve the WHO-recommended vaccination target in the country while contributing to achieving zero human deaths due to dog rabies by 2030.

In conclusion;

Among the control measures, dog vaccination plays a major role in preventing human rabies deaths in Sri Lanka. A team effort of relevant organizations is a must to achieve the recommended vaccination coverage while adhering to the revised vaccination schedule using anti-rabies vaccines of good immunogenic capacity. This makes rabies elimination an achievable target for Sri Lanka.

Collaborators

Dr Ruwini Pimburage who completed the first PhD project on rabies in the country.

Dr Hasanthi Rathnadiwakara is involved in the new project on immunogenicity and potency of anti-rabies vaccines marketed in the country.

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