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FOREWORD

The 7th KDU International Research conference 2014 on the theme Expanding Developmental Horizons through Education, Research and Innovation was organized by General Sir John Kotelawala Defence University on 21 and 22 August 2014. The expansion of KDU in education, research and innovation under the leadership of the Vice Chancellor, Major General Milinda Peiris has brought up the university into a leading university in the country. The university comprises of nine faculties including the faculty of post graduate studies and there is a separate Dean for the enhancement of the research culture in the university. KDU has taken many steps to boost the research culture of the university. The KDU International Research Conference (KDU-IRC) is one of the prominent events organized in every year making a great platform to national and international researchers to publish their research work.

The event included six plenary sessions under disciplinary sub categories, each organized under a sub theme followed by technical sessions in each discipline. The inaugural session was attended by eminent personalities such as Foreign Diplomats, Dr Sarath Amunugama, Senior Minister of International Monetary Cooperation & Deputy Minister of Finance & Planning, Tri-Service Commanders, Additional Secretaries, Chairperson of the University Grants Commission, Vice Chancellors and distinguished academics from other universities, representatives from countries like China, India, and Pakistan, Bangladesh and Maldives, and senior tri-service officers.

The inaugural session was followed by the plenary session on Defence held on the subtheme, Expanding Developmental Horizons through Education, Research and Innovations. The session was chaired by Prof. Rohan Gunaratne, Head of the International Centre for Political Violence and Terrorism Research (ICPVTTR) at Nanyang Technological University, Singapore. The plenary session on Medicine on the theme of Meeting the Health Needs of Sri Lanka through Education, Research and Innovation, was co-chaired by Professor Nimal Senanayake, Emeritus Professor of Medicine, Consultant Physician/Neurologist and Professor Janaka de Silva, Senior Professor and Chair of Medicine, Faculty of Medicine, University of Kelaniya. The plenary session on Allied Health Sciences was held on the theme of Education, Research and Innovation for expanding Horizons of Allied Health Sciences, chaired by Prof Narada Warnasuriya, Senior Professor, Faculty of Medicine, KDU and one of former vice chancellors of University of Jayawardanepura. The Engineering Plenary Session held on the theme, Achieving Development Goals through Research & Innovation in Engineering was chaired by Mr. W.J.L.S. Fernando, President, Institution of Engineers Sri Lanka. The plenary session on Law was conducted on the sub theme, Law and Development in 21 century. It was chaired by His Lordship, Honorable Mohan Peiris, the Chief Justice of Sri Lanka, The plenary session on Management, Social Sciences and Humanities was held on the sub theme, Sustainable Development through Integration of Multidisciplinary Knowledge, and the session was chaired by the Chairperson of the University Grants Commission of Sri Lanka, Professor (Mrs) Kshanika Hirimburegama.

KDU research conference has become increasingly popular among researchers in diverse fields of expertise, and it is inspiring to note that the response for our call for research papers was highly encouraging. This year, the committee received more than three hundred submissions of abstracts, which enabled us to select just above 175 high quality papers to be presented at the conference. All the submitted abstracts and papers were selected through a genuine double-blind reviewing process conducted by qualified academic and professional experts. We are grateful to the expert panel of reviewers, who took enormous effort to screen papers of high quality to be presented at the conference.

Because of the large number of papers committee decided to publish proceedings in six separate books in respective discipline. This book of proceedings contains all the plenary presentations either as full papers or as transcripts and the technical session papers presented at the conference in respective discipline. This publication is the finale of the success of the KDU International Research Conference 2014 and hence it is necessary to express our gratitude to all those who contributed for its success in numerous ways. First and foremost, I wish to extend my thanks and sincere gratitude to the Chairman of the Board of Management of
KDU, Mr. Gotabaya Rajapaksa and all the members of the Board. The support and the guidance of the Board of Management of KDU have extended to KDU are indeed immeasurable.

Organizing a gigantic task of this nature would be impossible without proper leadership and teamwork. Therefore, I would like to express my sincere thanks to the Vice Chancellor, Major General Milinda Peiris, for the excellent leadership provided during the conceptualization, planning and execution of the conference; and to the Deputy Vice Chancellor (Defence and Administration) Brigadier Lal Gunasekara for his invaluable contribution as the steering committee president that ensured the smooth execution of this event, and to the Deputy Vice Chancellor (Academics) Senior Professor Susirith Mendis for his guidance in finalizing papers.

Furthermore, I wish to acknowledge the services rendered by all the Deans of the faculties, the Heads of organizing committees and their teams, faculty representatives, Adjutant, CO Administration, Registrar, Bursar, Deputy Registrar, Assistant Adjutant, Assistant Registrar of faculty of Management Social Sciences & Humanities and all other academic and administrative staff members. They ensured the availability of some of the best national and international experts as plenary speakers, coordinated the tedious and time consuming review process thus ensuring better quality papers for publication and organized the event in a grand level.

I also thank the technical session rapporteurs who provided us with the summaries contained herein. In addition I would like to thank the Conference Secretaries Thilini Meegaswatte and Dilani Perera on their assistance during the organizing work of the conference.

Finally, I appreciate the role played by Wing Commander Jayalal Lokupathirage, the Head of the Department of Aeronautical Engineering for his commitment in organizing and coordinating all printing work of the conference including this book of proceedings.

Dr. RMNT Sirisoma
Editor
WELCOME ADDRESS

Major General Milinda Peiris RWP RSP USP ndc psc MPhil PGDM
Vice Chancellor, General Sir John Kotelawala Defence University

Distinguished members of the audience, ladies and gentlemen, it is my greatest pleasure as the Vice Chancellor of General Sir John Kotelawala Defence University to deliver the welcome and introductory address of the KDU international Research Conference 2014.

First, I am greatly honoured and pleased to welcome Hon Dr Sarath Amunugama, Senior Minister of International Monetary Cooperation and Deputy Minister of Finance and Planning, who accepted our invitation to grace this occasion as the chief guest and to deliver the keynote address on our theme “Expanding Developmental Horizons through Education, Research and Innovation.” Sir, I am sure that your address will set the right tone for the deliberations of this international research conference, which will be of immense significance for Sri Lanka as well as for other countries that seek development through education, research and innovation.

Next, I warmly welcome Commander of the SL Army, Lt Gen Daya Ratnayake, Commander of the SL Navy, Vice Admiral Jayantha Perera, Commander of the SL Air Force, Air Marshal Kolitha Gunatilleke, Additional Secretary Civil Security and Development Mrs Damayanthi Jayarathne, Treasury Representative Mr Mahinda Saliya, Your Excellencies of the Diplomatic Corps, Senior Officers, the Chairperson of the University Grants Commission Prof Kshanika Hirimburegana, Vice Chancellors, distinguished scholars and eminent personalities especially those representing our friendly countries such as the world renowned scientist Prof (Dr) Atta-ur-Rahman from Pakistan, Maj Gen PS Mander from the Indian Army, Maj Gen MD Zahidur Rahman from Bangladesh Army, Maj Gen SM Saffudeen Ahamad, Director General Bangladesh Institute of International and Strategic Studies (BIISS) and Dr Smruti Pattanaik (IDSA), Institute of Defence Studies and Analysis of India. And I warmly welcome all the presenters and participants whose contribution will be crucial for the success of the KDU Research Conference 2014.

Ladies and gentlemen, five years after the dawn of peace, Sri Lanka is steadily marching towards development and economic prosperity under the stable and inspiring stewardship of His Excellency the President Mahinda Rajapaksa. The country is no more groping in the dark; instead it is following the clear and sound vision encapsulated in *Mahinda Chinthana: The Way Forward*, which gives foremost priority for education, research and innovation as essential elements for sustainable development. It is in this backdrop that KDU has deeply pondered over its role and function as a university and taken many initiatives to introduce some crucial and innovative changes in higher education to complement the nation’s attempt to expand developmental horizons through education, research and innovation.

KDU has proved that nothing is impossible if done in the right spirit, dedication and commitment. So our innovations in the field of education have paid dividends for the country. A few years ago, the scope of KDU was to produce graduated officers for the armed forces. In contrast, today, its outlook has undergone a vast change, and it proudly caters to the nation’s higher educational needs by providing opportunities for civilian day scholars to follow high quality degree programmes in a disciplined environment. Today, we offer every opportunity for Sri Lankan as well as foreign students to become well-sought-after graduates in diverse fields. The target is to produce practically oriented, well disciplined, and patriotic graduates with soft skills essential for enhanced employability.

We have also undertaken a few mega projects that directly impact the expansion of educational opportunities in the country. The establishment of the southern campus of KDU at Sooriyawewa in Hambantota targeting the introduction of some unique degree programmes in the fields of Built Environment, Spatial Sciences etc is a major undertaking targeting expansion of educational opportunities to the periphery thereby ensuring parity in higher education in the country.
KDU hospital, the first ever university hospital in Sri Lanka with facilities for military and civil patient services, clinical trials and high-end laboratory testing, will be ready by August 2015, and it will provide assistance in healthcare for nation building. The establishment of the Faculty of Allied Health Sciences of KDU is yet another innovation that helps Sri Lanka to produce nurses, physiotherapists, and other allied health professionals whose service in developing the nation would be of considerable value.

Ladies and gentlemen, the conventional wisdom informs us that education expands horizons of knowledge which in turn facilitates innovation, and thus the rationale for expanding educational opportunities in a country. KDU having clearly understood its responsibility in this regard works as an agent of change in several significant areas in tertiary education in Sri Lanka. Accordingly, we have dedicated ourselves to linking education with research and innovation through several important projects, programmes and initiatives. For instance, KDU is developing its scientific research wing under KDU Institute for Combinatorial Advanced Research & Education known as KDU-CARE. The objective is to discover, implement and operate state-of-the-art scientific technologies which would enhance KDU’s scientific education and medical research capabilities.

KDU-CARE is progressing well and by now it has commenced 11 scientific projects as public private partnerships. We are in the process of establishing KDU-CARE Technology Park in KDU Southern Campus in Hambantota. Moreover, 4 factories – 2 in Sooriyawewa, one in Sewanagala and another in the hill country – will be set up in the coming months. We have attracted significant amount of funds as investments for these ventures. For the first time in Sri Lanka we will be constructing a Good Manufacturing Practices (GMP) certified manufacturing plant for producing antibodies and vaccines especially focusing on the production of anti venom for Sri Lankan poisonous snake bites. Further, we have introduced a Dean Research position first time in Sri Lanka to invigorate the research culture in the university. We have established a 24’X7’ research laboratory and a state-of-the-art advanced proteomics and genomics laboratory to facilitate the conduct of high-end research at KDU.

We have also introduced attractive incentives to encourage conduct of research, publications in high impact peer reviewed journals, and applications for patents. We believe that these measures we take today will definitely pay dividends for Sri Lanka in the near future.

The initiative taken by the Faculty of Graduate Studies to offer opportunities to read for research degrees at Master’s and PhD levels and KDU CARE are expected to generate high caliber researchers required for the country and its development.

Ladies and gentlemen, I make this an opportunity to make the humble claim that, all in all, KDU is playing a leading role in expanding higher educational opportunities in Sri Lanka thereby making a considerable contribution to make Sri Lanka a knowledge hub in Asia as envisaged in \textit{Mahinda Chinthana}.

Ladies and gentlemen, I should also express our grateful thanks to HE the President and his government for their blessings and encouragement for our initiatives; to the Secretary Defence, Mr Gotabaya Rajapaksa who is the tower of strength for KDU for his invaluable guidance in his capacity as the Chairman of the Board of Management, KDU; Commanders of the Army, Navy and Air Force, Additional Secretary, Civil Security and Development, and the Treasury Representative as members of the Board of Management for their diverse contributions for the upliftment of KDU.

Ladies and gentlemen, KDU’s annual international conference is a major contribution to the creation and dissemination of knowledge. The conference links other universities both local and foreign, higher educational institutions, professional bodies and the industry by providing a platform for their academics and professionals for presentation and publication of research in many fields of specialization.

It is heartening to note that this year the conference secretariat received an overwhelming number of abstracts out of which 195 were selected for presentation through a thorough peer reviewing process. Let us look forward to participating in highly fruitful plenary and technical sessions that will be held today and tomorrow at KDU.
Finally, let me conclude by once again welcoming all the dignitaries, intellectuals, and participants foreign and local and wishing that the deliberations of this conference would mark a positive contribution for the betterment of Sri Lanka, as well as the rest of the world. I conclude by wishing that KDU international Conference will be a fruitful and memorable one for all presenters and participants alike.

Thank you.
I am most grateful to the Vice Chancellor of KDU for inviting me to be here this morning and to speak to you on the subject of the development paradigm and education and science and technology. As we all know, this is a very important subject and we in Sri Lanka have quite a lot of experience in terms of innovation in education and trying to relate education to development. I want to begin with Millennium Development Goals. You know that heads of state met several years ago at the United Nations and identified 15 basic goals which they call the Millennium Development Goals. Which were to be achieved by 2015. That was the basis of what was called the Washington Consensus. While the developed countries would find funding for growth in developing countries, the developing countries, or the leaders of the developing countries has pledged to make every effort to reach those developmental goals among which were education and health. I am happy to say that of all the countries that were involved in this MDG exercise, after all we are all close to 2015 and we can evaluate whether we have succeeded in reaching those goals or not. Sri Lanka figures as a very good performance; gets very high marks in that scorebook for achieving the MDGs. So we have something to offer and discuss, when it comes to the contribution made by social welfare, by education, by health, to the development paradigm.

We must look at some of the theoretical arguments regarding what is called comparative advantage, that is in the globalized economy, when we think of economic development or economic growth, we have to analyze of all the factors and advantages that a country has, how do we position it in terms of comparative advantage. Different countries have different advantages. So in order to stimulate growth, we have to somehow identify those assets, those characteristics which give us comparative advantage as against rival economies or as against other competing economies. Now I want to refer (which I had also referred to at the last Defence Seminar) to the work done in the World Bank by the research division of the World Bank, where they are talking of what is called new structural economics. And what is the basis of that. The first is that today we are in a globalized economy. We cannot think of the national economy if we want to make the country rich and acquiring wealth that all the people in that country can benefit from that acquisition of wealth, which is reflected in technical terms as GDP growth. Now in Sri Lanka, we have a very enviable record, because after the end of the war a little over five years ago, we have had a very attractive GDP growth between 7-8 percentage points, which compares very favourably, not only with the earlier leaders of global growth, namely China and India but also with the traditional developed countries. As you know we are just emerging from a global downturn, global economic crisis, which first hit America, then the European Union countries, and subsequently impacted on the economies of China, India, Vietnam and so on, which were largely dependent on trade with these advanced economies.

Now in that context, Sri Lanka has done extremely well, averaging 7.5 to 8 percent growth and is now a member of the consortium of countries which are now driving global growth. The IMF and World Bank have estimated global growth to be in the region of 4 percent. Here, we are consistently reaching 7 to 8 percent growth which is very high. Now how do we maintain that, what is the basis of that. So one is our integration with the global economy. That is number one. Number two, is as I mentioned, to try to see within that global economy, what are our specific strengths and what is our comparative advantage as against rival economies.
advantage. Number three, is to see what is the role of the state in this growth process, particularly in relation to comparative advantage. Now in the past, there have been different approaches. Some have felt that only the state should be doing this in the past. That was not much of a success, because you saw that very many state ventures were not profitable, it dragged down the economy, and then there was a shift to the open economics. It sometime went to the other extreme. For example, even after the fall of the Soviet Union, Harvard University Professors were prescribing how the Russian economy should be tailored. It came to such almost ridiculous situation where free market economies were prescribed for the whole new Russian state because by that time the Soviet Union had disintegrated, the blueprints were being made in the Harvard University. So that is the other extreme. But today, we are looking at the interface. What is the role of the state, and what is the role of the private sector, and how can they jointly look into or assist in the growth process. So that is the third element. What is the mix between the state and the private sector. Now in the fourth area, we are looking at some aspects of how the state can make a distinct contribution together of course to help the private sector come into the picture. And that is called the provision of infrastructure, of two classes. One is what is called hard infrastructure, and the other is called the soft infrastructure. I am just giving a very broad definition. Hard infrastructure is that you cannot really think of benefiting from the globalized economy, unless you have roads, ports, airports, guaranteed energy. All those basic ingredients that will make your economy competitive. Now if you take South Asia, in a way I am sorry to say that only Sri Lanka can guarantee 24/7 power supply. All other countries in our region have power shedding. You cannot get power all the time. We too went through that period some time ago when we had to tell the investors you better bring your generators along.

That is not a very nice way of greeting an investor. But today Sri Lanka has achieved a situation where we are in a position to guarantee a continuous supply of power and we are hoping that over a period of time to make power cheaper. Then we have invested very heavily on roads, ports and airports, so one aspect which is not the focus of this seminar but very important is the investment in hard infrastructure that is contributing to our growth and that is now universally recognized as a very positive development in Sri Lanka in the Post-War period. Because we lost the possibility of expanding the physical infrastructure for thirty years. When a country is engaged in a destructive war which we won thanks to our President and to our distinguished army and other services represented here, where we were able to defeat terrorism comprehensively and catch-up on that thirty year lacuna. But that is not the subject of discussion in this seminar.

In this seminar we are talking about soft infrastructure. A country must also engage in creating the soft infrastructure that is necessary for growth. That is in this development paradigm of comparative advantage and many other things. In the earlier seminar I spoke about our global positioning and our geostrategic position, so we need not go into that today. So we are thinking of our comparative advantage in a globalized economy where the state and the private sector works together and now when we think of infrastructure we are going now into the area of soft infrastructure which means a modern educational system that can help in the growth process. So that is where we have to look at the question of education. Now, I think it is more than obvious to all of us, and certainly what we learn from professional educationists, is that we must move towards a scientific culture. The modern world of today and of the future will move towards a scientific culture. So our educational system has to be geared to providing the wherewithal and the investment to create a scientific culture in our economy. If we are not willing to do that and make the necessary investments our growth process itself will be impeded.

If you look at the Sri Lankan experience in education you can see that we have been moving towards it, but moving rather slowly. In the first phase Sri Lanka has a very enviable record because from the 1930s we have been emphasizing the role of education. Before independence in the State Council high priority was placed on providing education not only in the urban centres, which is the normal fashion in which education develops in most other countries, but education which spread out also to the rural areas. We generally call all that the Kanangara reforms or the Free Education Reform, Sri Lanka underwent very progressive educational reforms in the late 1930s and early 40s when what was called the Free Education System was set up. There were three pillars to that
educational system which we have now forgotten. One is, education free of cost, where all Sri Lankans had the ability to go to a school, a state funded school, or private schools at that time which got government assistance, like the Buddhist Theosophical Society, and they could get expect to get an education at least up to secondary level free of charge. But that was only one ingredient in that package the second ingredient was education in the English language. That was part of the reforms. Third, was what was called the Central College System, where there were residential colleges dotted throughout the country where bright children could attend and in any large audience in Sri Lanka you will have many people who have built their career on that system.

However, at a certain stage pressure was created on the educational system by population growth. The present government has had to revamp that whole educational system where you push the educational system towards a science culture. That is what you now call the Mahindodaya System where the government is going to reorganize the whole educational structure where the country will be dotted with a large number of science colleges with dual languages and a large investment in Information Technology. This requires a large investment which is going to take some time. This requires a public-private partnership which delivers the necessary services to the people. This entire transformation has to be driven by a scientific culture.

When we were young I think Daya and all will remember, it was very difficult to get a telephone. When you are a young officer it was almost impossible to get a telephone, you have to go to so and so and so on. But today within ten minutes you will get the mobile phones. There are 220 lakhs of telephone circulating in this country. That is what you now call the Mahindodaya System where the government is going to reorganize the whole educational structure where the country will be dotted with a large number of science colleges with dual languages and a large investment in Information Technology. This requires a large investment which is going to take some time. This requires a public-private partnership which delivers the necessary services to the people. This entire transformation has to be driven by a scientific culture.

If you look at the agricultural sector, we cannot get people to go back to the same old system of agriculture. You take buffalos or have a blown or going in a spinlock I mean that is simply not possible. And as a result what is happening is that in real terms our agricultural is expanding, but in terms of share of GDP it is being reduced. That is a normal phenomenon. As country grows the agriculture sector shrinks in relation to GDP. In real terms it expands, but more wealth is being created in the manufacturing and the service sector. Our service sector very much like tourism, like construction, like infrastructure development, financial services are expanding in terrific way. We want to make Sri Lanka a hub in Asia and that is our next step. To make it a knowledge hub we have to make it to a joint enterprise. Earlier the state had to do everything but today state is not a position to make all these investments. So it has to be shared. I’m very glad that the chairman of the UGC is also here, because somewhere it has be shared, because we have to confront that problem.

Lastly, many of our young people go to foreign universities. Our education system is better than the education systems of some of these countries. Lots of middle class people are spending money to send their children abroad. This is because the state system has not expanded enough to include these people. Now as we just heard from the Vice Chancellor of this university there are other new areas where we will have to expand our tertiary and university education to gather this vast potential of scientific oriented people who will otherwise be utterly frustrated prevented from doing their higher studies because of policy difficulties. So we have to change the policy to make Sri Lanka an education hub and that is the next step to be undertaken.

While we have a very innovative garment sector and are going up to the top end of the garment industry in the process of becoming a middle income country we did not get the GSP + , the preferential tariffs which were given to certain countries, particularly by the European Union. The US preferential tariffs still remain. But our garment industry has adapted itself. It is very good example of modern technology being used, high-end scientific technology, and today, though of course it is a component of a global textile industry. Ours is a highly innovative up market garment industry. I think all of you have visited some of our malls here, I don’t want to mention names because that will
give an unfair commercial advantage, but you have a lot of malls here and you can see the quality of the products. The cost is very reasonable and the product is of a high quality, that is the type of garment industry that we have developed. It is a US$ 10 billion enterprise. Then we also have tourism. It is also growing very fast. By next year we expect 2.5 million tourists to come in to this country. These are all in the service sector which requires new thinking, innovation, these are new areas which will also impact our domestic sector. Even in agriculture we cannot think of sustaining agriculture unless we can modernize with new equipment. Recently we had a discussion in parliament about the eastern and northcentral provinces, and about how they have brought in a new harvester. Because earlier where they used very simple mechanisms now with this new mechanism they can do a much more efficient job. So that sort of innovation is needed. Earlier we had to bring workers from India to handle that machine but now Sri Lankan young people in villages are able to mange that large scale harvester. So that type of scientific and technological culture has to be created. Particularly in a small country where we have to go for niche markets and innovate, we have to create an educational system and space for a general growth pattern for this new scientific community. So I think that we are going through an interim phase. We are completely revamping our education system, still we are at the level of primary education and basic education but as we go along it will impact our secondary education, tertiary and then university education and like that it will go along. So once you set this new curriculum and new structure, once you set it loosely, obviously it is going to have an impact on higher classes in the years to come. So we have to plan for that. So that is what I see in the new development paradigm. A key role will be played by science and technology and by education because it will be a link to whatever comparative advantage that comes to us by the nature of our physical positioning, by the nature of the markets, by the nature of human capital, by the nature of government investments, and all those other variables. Science and technology will play a key role in positioning the country in a competitive stance in a highly global economic situation.
PLENARY SESSIONS

Meeting the Health Needs of Sri Lanka through Education, Research and Innovation
PLENARY SESSION SUMMARY

The medical plenary session was chaired by Professor Nimal Senanayake, Emeritus Professor of Medicine Consultant Physician/Neurologist and former Senior Professor of Medicine and Dean, Faculty of Medicine, University of Peradeniya. Four eminent guest speakers were invited to address this session under the theme of “Meeting the health needs of Sri Lanka through education, research and innovation”.

Prof. Chandrika Wijeyaratne, Prof. Gamini Goonetilleke, Prof. Athula Sumathipala and Dr. Ravindra P. Rannan-Eliya were invited as the plenary session speakers.

Professor Chandrika Wijeyaratne was the first speaker in the Medical Plenary Session. During her presentation on “NIROGI Lanka Project: A pragmatic approach to tackling chronic Non Communicable Disease (NCDs) in Sri Lanka”, she discussed about how Nirogi Lanka Project acts to improve primary care, screening and prevention to control the risk factors of NCDs in the country. She mentioned that the project “National Initiative to Reinforce and Organize General diabetes care In Sri Lanka (NIROGI Lanka)”, with funding from the World Diabetes Foundation, was initiated in 2009.

Prof. Gamini Goonetilleke was the second speaker of the session and in his presentation on “Trauma care and control in Sri Lanka: the need for education and research” he pointed out that, trauma is a major cause of death and disability throughout the world and in Sri Lanka trauma is the leading cause of admissions to hospitals. He mentioned that road traffic accidents (RTA), occupational injuries, war injuries, burns, shotgun injuries, chemical injuries etc. contribute largely for major trauma cases. He specifically pointed out that number of RTAs have increased largely since 1991. Further, he highlighted that the facilities currently provided for trauma care are inadequate, thus there is an urgent need to improve facilities for trauma care and to educate on the prevention of trauma.

Prof. Athula Sumathipala delivered a presentation on “Research capacity and infrastructure development as a part of nation building”. In his presentation he stated that research funding dedicated to health problems in developing countries is not adequate. During his presentation he talked about establishing research capacity and excellence at individual, group, institutional and national level which will improve the health sector. He encouraged strong collaborative research partnerships between developed and developing countries and also collaborations between developing countries with a robust ethical and governance framework.

Dr. Ravindra P. Rannan-Eliya made a presentation on “The importance of local research in developing health strategy – The case of cardiovascular disease prevention in Sri Lanka”. He mentioned that age-sex specific IHD mortality rates are substantially higher than in developed countries, and therefore it is essential to reduce IHD mortality rates to make a substantial progress in countering the NCD epidemic and to maintain improvements in life expectancy. He pointed out that the current strategy in Sri Lanka can be improved by revising national protocols and using local research data.

Concluding the session, Prof. Nimal Senanayake appreciated the presentations and highlighted the need of development of research in order to develop the health sector in Sri Lanka.
NIROGI Lanka project: a Pragmatic Approach to Tackling Chronic Non Communicable Disease (NCDs) in Sri Lanka?

Prof. Chandrika Wijeyaratne, Dr. Kayathri Periyasamy, Dr. Noel Somasundaram, Dr. Carukshi Arambepola

I. BACKGROUND

Reducing premature death and disability caused by chronic NCDs, chiefly cardiovascular risks and diabetes, is the decade long priority of the Diabetes Prevention Task Force established of the Sri Lanka Medical Association. Multi-stakeholder involvement of health policy makers and planners to practicing clinicians, academics and administrators in the state and private sectors is a key feature.

The project National Initiative to Reinforce and Organize General diabetes care In Sri Lanka (NIROGI Lanka), with funding from World Diabetes Foundation, was initiated in 2009.

II. PROJECT OBJECTIVES

- To initiate a national programme for capacity building in diabetes care by establishing a pioneer cohort of ‘diabetes nurse educators’ (DENO) in Sri Lanka
- To improve the quality of diabetes care by establishing a health care model of tertiary-primary care partnerships in Colombo by upgrading selected primary care curative sector institutions and monitoring their protocol based patient management, under the supervision of the tertiary care
- To strengthen the community through health promotion in impoverished, overcrowded urban settings that aim at primary prevention, screening and risk reduction

III. PROJECT OUTCOMES

- Training of 279 Diabetes Educator Nursing Officers (DENOs) for the state sector hospitals and coordinating the provision of holistic care for diabetes and CVD risk care throughout Sri Lanka; coupled with appropriate training of related health professionals (74 Health Education Nursing Officer and 64 selected private sector Nurse aids)
- Tailor made training of 119 MO/RMOs and 69 family practitioners who function in primary care settings in the western province
- Strengthening laboratory services for managing diabetes and cardiovascular risks at primary care centralised at Maligawatte DH
- Infrastructure development of primary care health services in 6 selected settings that provided service for 109,947 patients (in 18 months) in Western Province through a partnership between the tertiary care and the primary care services. Additionally screening of 25,836 adults was carried out through 526 screening camps in the community and work settings.
- Empowering the community with family participation in Kotte and Kolonnawa through 133 health promotion settings located at workplaces, schools and community centres through capacity building of health workers and Health Promoter that encourages positive behaviour

IV. FINAL OUTCOME

Disseminated the evidence generated to advocate policy makers to replicate this approach island wide. Invitation by WDF to apply for second phase – which is now mid term and addresses NirogiMaatha (pregnancy diabetes), NirogiPaadha (diabetes foot care) and NirogiDiviya (health lifestyle)


V. PROJECT OUTPUTS TO DATE

- Training Manual for Diabetes Educator Nurses
• Handbook for Health Educator Staff
• Hand Book for Primary Health Care
• Cardiovascular Disease
• DVD on Nutrition for a Healthy Life
• DVD on Exercises for a Healthy Life
• Health Education tools including food pyramids, food table to educate on portion sizes with a formatted arrangement for outdoor tent display
• Personal health record for clinic use
• Diabetes in Pregnancy (IEC material Sinhala & Tamil) - ISBN 978-955-9386-27-8
• DVD on Diabetic foot care (Sinhala & Tamil)
• DVD on physical activity in Pregnancy
• Flip charts on Diabetic Foot care Step by Step & Doctor Paadhathana
• Booklet on Diabetes & your Feet by Dr. Thusitha Kahaduwa
• Guideline for Screening, Diagnosis & Management of Diabetes in pregnant women
• Leaflets –
  ➢ 1-7 leaflet set on What is Diabetes
  ➢ What is Heart attack
  ➢ What is Cholesterol

➢ Use of coconut & coconut products

• Stickers – High Risk Foot & Low Foot risk for risk categorization in clinics
• Posters – Diabetes Prevention
  Health Awareness Flex Banners
  Poster competition wall & desk Calendar
• Bags & NIROGI Diviya Badges
• Monofilaments - Tester for Diabetic foot examination
• NIROGI Pens

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• Pocket Guide to Diabetic Foot Care ISBN 978-955-30-2193-9 by Prof. Mandika Wijeyaratne
• Proper care for healthy feet ISBN 978-955-30-2192-2 by Prof. Mandika Wijeyaratne
• Diabetes & your child ISBN 978-955-0629-01-5 by Prof. Shamya de Silva

WEB LINK
http://nirogilanka.org/
ORAL PRESENTATIONS
Tuberculous and Toxoplasma Lymphadenitis in Lymph Node Biopsies Received at THP Laboratory from 2011 to 2013

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Abstract—
Background—Lymph node biopsy is an important investigation which is helpful in coming to a diagnosis when patients present with lymphadenopathy. Tuberculous and Toxoplasma lymphadenitis are frequent findings in lymph node biopsies done in Sri Lanka compared to developed countries as both of these conditions are commoner in developing countries. Therefore we planned to review the lymph node biopsy results from 2011 to 2013 in Teaching hospital Peradeniya with a special interest on clinico-demographic characteristics of tuberculous lymphadenitis and Toxoplasmosis. However histopathological findings can only give a suggestive diagnosis of toxoplasmosis, whilst confirmation needs serological studies.

Objectives—To determine the incidences of histopathologically proven tuberculous and Toxoplasma lymphadenitis and the other commoner pathologies out of the lymph node biopsies done in THP from 1st January 2011 to 31st December 2013 and to identify the clinico-demographic characteristics of the former two conditions.

Methods—Lymph node biopsy reports and the request forms from 2011 to 2013 in THP were reviewed. Age, sex, site of lymphadenopathy and the pathological diagnoses were tabulated and analyzed using SPSS.

Results—Out of 293 lymph node biopsy reports, 47 (16%) showed tuberculous lymphadenitis, 16 (5.5%) showed toxoplasma lymphadenitis, 75 (25.9%) reactive changes and 54 (18.4%) lymphoma. Metastatic deposits were found in 33 (11.3%). With regard to tuberculous lymphadenitis, females (59.57%) were affected more than males (40.43%). A similar pattern was observed with 63% of those affected being females. In both of these conditions the commonly affected age group was 21-30 years. The most commonly involved group of lymph nodes was cervical (78% of the TB cases and 100% of the cases of toxoplasmosis). The 2nd commonest affected site was axilla.

Conclusion—The incidences of both tuberculous lymphadenitis and toxoplasmosis are high. There is a female preponderance in incidence and the 21-30 years age group is commonly affected with cervical lymph nodes being the commonest site. However the commonest pathology affecting lymph nodes is reactive changes in this group.

Keywords—tuberculous lymphadenitis, nodal toxoplasmosis, lymph node biopsy

I. INTRODUCTION

Peripheral lymphadenopathy may indicate various underlying pathologies and it has continued to pose a diagnostic problem in medical practice throughout time. These patients who are clinically diagnosed with lymphadenopathy usually need investigations to arrive at a definitive diagnosis. Of these, most often histopathological analysis of the lymph node tissue has the final say in diagnosis, except for serological studies and PCR with regard to infections.

The aetiology for lymphadenopathy may vary from non specific infections to conditions like tuberculosis and toxoplasmosis and to more grave pathologies like malignancy.

Of these infections, tuberculosis is documented to be a commoner cause in tropical countries while malignancies are reported as the predominant cause of lymph node enlargement in the developed countries with the rarity of infections (Tiwari et al, 2008).

With Sri Lanka being a tropical country we have concentrated mainly on tuberculosis and
toxoplasmosis to describe their clinico-demographical pattern and incidences, in addition to certain other aetiologies.

Tuberculosis (TB) remains a major global public health problem. It is estimated that about one-third of the world’s population is infected with *Mycobacterium tuberculosis*. There were an estimated 8 million new cases of TB, resulting in 1.9 million deaths; with the greatest burden of disease in developing nations. Lymphadenopathy is one of the most common presentations of extra pulmonary tuberculosis as lymphatic system is a frequently involved. Tuberculous lymphadenitis occurs relatively early after primary infection with *M. tuberculosis* and often affects young people in countries with a high prevalence of tuberculosis (Eshete et al, 2011).

In the mean time, toxoplasmosis, a parasitic disease caused by the protozoan *Toxoplasma gondii* can be seen throughout the world (Ryan, Ray, 2004). The commonest presenting sign of acquired toxoplasmosis in man is the enlargement of superficial lymph nodes. Cats are the primary source of infection to human hosts, although contact with raw meat, especially lamb, is a more significant source of human infections in some countries with faecal contamination of hands also being a significant risk factor ((Ryan, Ray, 2004; Torda, 2001). It is also documented that up to a third of the world’s human population is estimated to carry a *Toxoplasma* infection (Montoya, Liesenfeld, 2004)

This study aims at finding causes of lymph node enlargement and their pattern of lymph node involvement with more focus on tuberculosis and toxoplasmosis in order to find more specific information relevant to our country.

II. OBJECTIVES

To determine the incidences of histopathologically proven tuberculous and *Toxoplasma* lymphadenitis and the other commoner pathologies out of the lymph node biopsies done in THP from 1st January 2011 to 31st December 2013. To identify the clinico-demographic characteristics of the first two conditions.

III. METHODOLOGY

This is a retrospective study of lymph node biopsies received by the department of Pathology, Faculty of Medicine, University of Peradeniya, from Jan 2011 to Dec 2013. Demographic and clinical data and the pathological diagnoses were obtained from the request forms. The lymph node excisions done as a part of malignancy treatment were excluded from this study.

With regard to Toxoplasma lymphadenitis, the presence of the following characteristic features have been considered in diagnosing; marked follicular hyperplasia, associated with intense mitotic activity and phagocytosis of nuclear debris, small granulomas composed almost entirely of epithelioid cells, located within the hyperplastic follicles and at the periphery, encroaching on and blurring their margins and distension of marginal and cortical sinuses by monocytoid B cells. The presence of immunoblasts and plasma cells in the medullary cords was also taken into account.

Similarly, biopsies with appearances ranging from multiple small epithelioid granulomas to huge caseous masses surrounded by Langhan’s type giant cells, epithelioid cells, and lymphocytes were taken as being positive for tuberculosis. Although Ziehl-Neelsen stain had been performed in these specimens, visible acid fast bacilli were not identified in any of them.

All these biopsy findings and other previously mentioned clinical and demographical data were tabulated and analysed using SPSS version 20 software.

IV. RESULTS

Of the two hundred and ninety three lymph node biopsies reviewed during the 3 year period (2011-2013) of study, 179 biopsies were from the cervical group of lymph nodes constituting 61.1% of all lymph nodes biopsies. Analysis of the data on these 293 patients showed that 19.4% and 18.7% were in the age group of above 60 years and 21-30 years respectively. The male to female ratio was 1:1.
Table 1. Histopathological diagnoses

<table>
<thead>
<tr>
<th>Pathological diagnosis</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculous lymphadenitis</td>
<td>47</td>
<td>16</td>
</tr>
<tr>
<td>Toxoplasma lymphadenitis</td>
<td>16</td>
<td>5.5</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>54</td>
<td>18.4</td>
</tr>
<tr>
<td>Reactive change</td>
<td>76</td>
<td>25.9</td>
</tr>
<tr>
<td>Metastatic deposits</td>
<td>33</td>
<td>11.3</td>
</tr>
<tr>
<td>Necrotizing lymphadenitis</td>
<td>20</td>
<td>6.8</td>
</tr>
<tr>
<td>Other</td>
<td>47</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>293</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 1 shows the histopathological diagnoses of lymphadenopathy as obtained in this study. Tuberculosis was seen in 47 (16%) cases, 16 (5.5%) showed toxoplasmosis, 75 (25.8%) showed reactive changes and 54 (18.4%) showed lymphoma while metastatic deposits were found in 33 (11.3%) cases.

An important pathological diagnosis in our study was reactive changes which was found to be the most common pathology found in lymph node biopsies, followed.Governments and Non-Governments, 2006. However, Metastatic deposits were seen only in 11.3% of the cases which is a very low incidence in comparison to a 65.0% incidence documented in the western series yet our incidence is in accordance to other Asian studies (Tiwari, 2008). This shows that metastatic deposits are more common in western countries where socioeconomic status is high and possibly due to the reduced incidence of tropical diseases like tuberculosis and toxoplasmosis. However, when lymphoma and metastatic deposits are taken together, the incidence is much higher than that of tuberculosis and Toxoplasma lymphadenitis indicating that we are also moving from infectious diseases towards non communicable diseases like neoplastic conditions.

The age range of patients with tuberculous lymphadenitis was 1.2 to 62 years with the peak age being the 3rd decade. Overall, the male to female ratio for tuberculous lymphadenitis was 2.3. The commonly affected site was cervical group of lymph nodes (78%).

Meanwhile, of the 16 cases with toxoplasmosis, the age range of the patients was 13 to 68 years with the peak age being the 3rd decade. Overall, the male to female ratio for toxoplasma lymphadenitis was 3:5. A marked decline in the incidence of toxoplasma lymphadenitis was observed after the 5th decade with 43.7% of cases occurring before the age of 30 years. The commonly affected site was cervical group of lymph nodes.

Neoplastic disease was seen in 87 (29.7%) cases, and this group constituted Hodgkin and non Hodgkin lymphoma as well as metastatic deposits. Overall, the pathologies of lymph nodes were seen to be affecting males and females equally. Those in their sixties or above were found to present more commonly with lymphadenopathy than young people and these mostly involved the cervical group followed by axillary group. But these figures were observed to differ with each individual pathology.

Tuberculosis was found to be high in our study but it was noted to be lower than the figures found in a similar Nigerian study (26.7%) (Adesuwa, Chibundu, 2006) and in a Nepal study (47.0%) (Tiwari, 2008). Both TB and toxoplasmosis showed a similar pattern of involvement, with being more prevalent in females than males and involving the cervical group of lymph nodes mostly. Also, they were seen to be more prevalent in the young age group (21- 30). In the mean time both lymphoma and metastatic deposits were prevalent in old age (>60 years) and in males. This pattern of disease was comparable to similar studies done in other countries (Olu-eddo, Omoti, 2011, Eshete et al, 2011, Daudpota et al, 2013, Tiwari, 2008, Durlach et al, 2003).

This shows that an importance should be given to those who present with lymphadenopathy with regard to tuberculosis and toxoplasmosis as misdiagnosis of these two can lead to unnecessary morbidity and mortality which could have been prevented with early diagnosis and treatment.

Although diagnosis based on histopathological features is not the gold standard in nodal
toxoplasmosis and tuberculous lymphadenitis, there is evidence to justify the use histopathology for diagnosing these two conditions. Previous studies have shown that toxoplasmosis lymphadenitis can be diagnosed using specific histopathological features with a high degree of confidence (Eapen, 2005) and in the case of tuberculous lymphadenitis, histopathology has shown a sensitivity of 96% and a specificity of 78.5% (Patwardhan, 2011).

In our study, Ziehl Neelsen staining has not shown acid fast bacilli in any of the tuberculous lymphadenitis specimens. This test needs the presence of more than 10,000 bacilli for it to be positive for tuberculosis. Also it has been proven in past studies that a negative result for this staining doesn’t exclude tuberculosis (Gupta, 1999).

One of the main limitations of this study was being unable to use more sensitive advanced molecular techniques like PCR and culturing method for the detection of *M. tuberculosis* and serological tests for toxoplasmosis in addition to histopathology as ours was a retrospective study.

VI. CONCLUSION

The findings of this study reveal that tuberculosis and toxoplasmosis are two common aetiologies for lymphadenopathy especially in the young female population of our country which need to be considered in clinical practice in order to target early diagnosis and proper treatment.

REFERENCES


BIOGRAPHY OF AUTHORS

¹Author is a consultant pathologist and a senior lecturer of the department of Pathology, faculty of Medicine, university of Peradeniya. Her research interests include neuromuscular diseases, gynecological pathologies and tropical medicine. She has produced many international and local publications to her credit.

¹²³ Authors are pre-interns who are currently working as temporary lecturers in the Department of Pathology, faculty of Medicine, university of Peradeniya.
Knowledge of Hand Hygiene among the Medical Students at the General Sir John Kotelawala Defence University

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Abstract

Background. Hospital acquired infections are commonly transmitted by the hands of healthcare workers. The objective of this study was to compare the knowledge of hand hygiene among the preclinical students and the students with clinical exposure.

Method. A self-administered, pre-tested validated questionnaire from previous publications, based on hand hygiene guidelines by the WHO, was distributed among all the medical students at the General Sir John Kotelawala Defence University. Results were analyzed by comparing the knowledge of the WHO guidelines with the appropriate hand hygiene behaviour of the medical students. The percentages were calculated and compared using the statistical test for proportions. A p<0.05 was considered to be statistically significant.

Results. All 104 (58.75%) preclinical students and 73 (41.24%) students who had clinical exposure participated in the study.

Of the preclinical students the percentage who knew that the hands should be washed before having direct contact with patients, after having direct contact with the patients, if moving from a contaminated body site to a clean body site in the same patient, before clean/ aseptic procedures, after contact with body fluid/blood and after contact with any object in patient's immediate surrounding were 67.30%, 50%, 50.96%, 88.46% and 34.61% respectively. Of the student with clinical exposure, these were 49.31%, 63.01%, 31.50%, 87.67%, 94.52% and 27.39% respectively.

Twenty-two (21.15%) of the preclinical students and 29 (39.72%) of the students with clinical exposure knew the correct duration of hand washing (p=0.007).

Conclusion. Correct duration of hand washing (p=0.007) and knowledge of hand hygiene before clean/ aseptic procedures (p<0.001) were significantly better among students with clinical exposure. The knowledge on other aspects showed no significant difference between the two groups (p>0.05). In general, the hand hygiene knowledge and practice among medical students was not satisfactory. Therefore, further education with regard to hand hygiene is necessary.

Keywords: Hand hygiene, Medical students, General Sir John Kotelawala Defence University

I. INTRODUCTION

Health-care associated infections continue to pose a serious threat of increasing mortality and morbidity among the hospitalized patients. World Health Organization reports that at any time, over 1.4 million people worldwide suffer from infections acquired in health-care settings (WHO guidelines on hand hygiene, 2005). The rates of nosocomial infections tend to be as high as 39% in hospitals located in resource-poor countries (Rizvi , et al., 2007).

Organisms that cause nosocomial infections are most commonly transmitted by the hands of healthcare workers (Kusachi, et al., 2006). Therefore, hand-hygiene is considered to be the single best measure for infection control and it has been observed that rates of nosocomial infection are considerably reduced when healthcare workers act in accordance with recommended guidelines for hand hygiene (Kusachi, et al., 2006, Lam, et al., 2004, Pittet, et al., 2000). Despite this fact, adherence to hand-hygiene practices remains consistently poor among the health care workers (Pittet, et al., 2000, Kaplan, et al., 1986, Danchaivijitr, et al., 2005). Notable factors for poor compliance include inaccessibility or shortage of hand-washing equipment (Kaplan, et al., 1986, Danchaivijitr, et al., 2005, Akyol, et al., 2007, Sax, et

Medical students are an integral part of the health care team. Therefore it is important to assess their knowledge of hand hygiene, to reduce the incidence of health care associated infections. To bring about a change it is necessary to first collect information about medical students’ assessment of their own behaviour towards hand-hygiene and their attitude towards possible interventions. Many studies in this domain have been carried out in the West (Graf, et al., 2012, Scheithaner, et al., 2011), but sparse data is available from developing countries.

The objective of this study was to compare the knowledge of hand hygiene among the preclinical students and the students with clinical exposure.

II. MATERIALS AND METHODS

Study design and setting: This study was a descriptive cross sectional study. The study was carried out at the Faculty of Medicine, General Sir John Kotelawala Defence University in September 2013.

Study population: All the medical students at the Sir John Kotelawala Defence University were participated in the study.

Presently there are five batches of medical students. The numbers of medical students are one hundred and seventy seven.

Data collection and data collection tools: Self-administered, pre-tested validated questionnaire from previous publications, based on the hand hygiene guidelines laid down by the World Health Organization (WHO guidelines on hand hygiene, 2005) was used for data collection and the questionnaire was administered in English. The questionnaire was distributed to all the 177 medical students after a brief explanation. It was explained to the students that participation is voluntary and that there will be no repercussions for not participating in the study. If they do not give consent they can return the incomplete questionnaire. Filling of the questionnaire implies consent. The students were requested to hand over the completed questionnaire to the Department of Para Clinical Sciences.

Statistical analysis: Results was analysed by comparing the knowledge of WHO guidelines with the appropriate hand hygiene behaviour of the medical students. The percentages were calculated and compared using z test for proportions. A p<0.05 was considered to be statistically significant.

III. RESULTS

All 177 students to whom the questionnaires were distributed participated in the study. Of the students 132 (74.58%) were males.

There were 104 (58.75%) preclinical students and 73 (41.24%) students who had clinical exposure.

Thirty (28.84%) of the preclinical students and 25 (34.24%) of the students with clinical exposure knew that hand washing is the single most important event in the prevention of hospital acquired infections (p=0.445).

The numbers and the percentages of the preclinical students who knew the importance of each component of hand hygiene is shown in Table 1.

Table 1: The numbers and the percentages of the preclinical students who knew the importance of each component of hand hygiene

<table>
<thead>
<tr>
<th>Component of hand hygiene</th>
<th>Number</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hands should be washed before having direct contact with patients</td>
<td>56</td>
<td>53.84%</td>
</tr>
<tr>
<td>after having direct contact with the patients</td>
<td>70</td>
<td>67.30%</td>
</tr>
<tr>
<td>if moving from a contaminated body site to a clean body site in the same patient</td>
<td>52</td>
<td>50%</td>
</tr>
<tr>
<td>before clean/ aseptic procedures</td>
<td>53</td>
<td>50.96%</td>
</tr>
<tr>
<td>after contact with body fluid/ excretion/ blood/ mucus membranes/ non intact skin/ wound dressings</td>
<td>92</td>
<td>88.4%</td>
</tr>
<tr>
<td>after contact with any object in patient’s immediate surrounding</td>
<td>36</td>
<td>34.61%</td>
</tr>
</tbody>
</table>
The numbers and the percentages of the students with clinical exposure who knew the importance of each component of hand hygiene is shown in Table 2.

Table 2: The numbers and the percentages of the students with clinical exposure who knew the importance of each component of hand hygiene

<table>
<thead>
<tr>
<th>Component of hand hygiene</th>
<th>Number</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hands should be washed before having direct contact with patients</td>
<td>36</td>
<td>49.31%</td>
</tr>
<tr>
<td>after having direct contact with the patients</td>
<td>46</td>
<td>63.01%</td>
</tr>
<tr>
<td>if moving from a contaminated body site to a clean body site in the same patient</td>
<td>23</td>
<td>31.50%</td>
</tr>
<tr>
<td>before clean/ aseptic procedures</td>
<td>64</td>
<td>87.67%</td>
</tr>
<tr>
<td>after contact with body fluid/ excretion/ blood/ mucus membranes/ non intact skin/ wound dressings</td>
<td>69</td>
<td>94.52%</td>
</tr>
<tr>
<td>after contact with any object in patient's immediate surrounding</td>
<td>20</td>
<td>27.39%</td>
</tr>
</tbody>
</table>

None of these differences were significant except if moving from a contaminated body site to a clean body site in the same patient (p=0.014) and before clean/ aseptic procedures (p<0.001). Twenty-two (21.15%) of the preclinical students and 29 (39.72%) of the students with clinical exposure knew the correct duration of hand washing (40 to 60 seconds) (p=0.007). Seventy-four (71.15%) of preclinical students and 56 (76.71%) students with clinical exposure said they need further education in hand hygiene.

IV. DISCUSSION

We compared the knowledge of hand hygiene among the preclinical students and the students with clinical exposure at the Faculty of Medicine, General Sir John Kotelawala Defence University, Sri Lanka.

Our findings indicated that the correct duration of hand washing (p=0.007) and knowledge of hand hygiene before clean/ aseptic procedures (p<0.001) were significantly better among students with previous clinical exposure. The knowledge on other aspects of hand hygiene showed no significant difference between the two groups.

A study which was conducted in a Turkish medical school in 2010 also indicated that the knowledge and practices of hand hygiene among the medical students were insufficient (Ergin, et al., 2011).

Although hand hygiene is a simple procedure, in general, the hand hygiene knowledge and practice among these military medical students did not meet the current standards set by the World Health Organization (WHO). Therefore, development of hand hygiene promotion programs will be necessary to improve the hand hygiene of our students.

REFERENCES


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**BIOGRAPHY OF AUTHORS**

1 Author is a lecturer in Medical Microbiology at the Faculty of Medicine, General Sir John Kotelawala Defence University, Sri Lanka. Her research interests are Mycobacteria, infection control and Management of outbreaks. She has presented papers in local and international conferences.

2 Author is a senior lecturer and specialist in Medical Microbiology of Faculty of Medicine, General Sir John Kotelawala Defence University, Sri Lanka. His research interests are Hospital associated infection, infection control and antibiotic resistance.

3 Author is a senior lecturer of the Faculty of Medicine, KDU and a senior consultant community Physician. His research interests are elderly, health promotion and NCD epidemiology. He is a Lieutenant Colonel of the Medical corps as a volunteer officer.
A community based Study on Nasal Carriage of *Staphylococcus aureus*

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Abstract

**Background.** *Staphylococcus aureus* is a major bacterial cause of superficial infections and a healthcare associated infection. The emergence of multiple resistances to anti staphylococcal penicillins like cloxacillin, methicillin and other agents has compromised therapy. Methicillin Resistant *Staphylococcus aureus* (MRSA) infection has become a major problem among the hospitals and the communities. Community studies in MRSA colonization are lacking. The aim of this study was to describe the prevalence of Methicillin Sensitive *Staphylococcus aureus* (MSSA) and MRSA nasal colonization in the community and antibiotic susceptibility patterns of these isolates.

**Methods.** This study was a community based descriptive cross sectional study. Nasal samples for *S.aureus* culture and sociodemographic data were obtained from 317 adults and children ≥2 years of age from the selected two communities over a period of two months. *S. aureus* isolates were identified by routine laboratory methods and antimicrobial susceptibility tests were done by following the CLSI guidelines.

**Results.** Of the 317 persons, 88(27.76%) were positive for *S. aureus*. Of the 88 isolates, 18 were MRSA and 70 were MSSA. Of the MRSA isolates the sensitivity to Erythromycin, Clindamycin, Linezolid, Ciprofloxacin, Trimethoprim-sulfamethoxazole, Tetracycline and Gentamicin were 5.55%, 33.33%, 100%, 94.44%, 94.44%, 77.77%, 94.44% respectively. Of the MSSA isolates the sensitivity to Erythromycin, Clindamycin, Cefoxitin, Linezolid, Ciprofloxacin, Trimethoprim-sulfamethoxazole, Tetracycline and Gentamicin were 44.28%, 78.57%, 100%, 100%, 72.85%, 95.71%, 84.28%, 81.42% respectively. Inducible Clindamycin resistance was reported 44.44%, 7.14% for MRSA and MSSA isolates respectively.

**Conclusion.** More than a quarter of the study population were colonized with *S. aureus*. MRSA colonization prevalence was 5.67% (18/317). More than 75% MRSA isolates were sensitive to Ciprofloxacin, Gentamicin, Trimethoprim – sulfamethoxazole, Tetracycline, Linezolid. Therefore, MRSA isolates of this study were more likely to be community acquired. However, further molecular studies are needed to confirm these findings.

**Keywords:** Methicillin resistant *Staphylococcus aureus*, *Staphylococcus aureus*, community-acquired.

I. INTRODUCTION

*Staphylococcus aureus* is the major bacterial cause of skin, soft tissue and bone infections and one of the commonest causes of healthcare associated infections. Antibiotics are the basis for treatment of staphylococcal infections, but the emergence of multiple resistances to anti staphylococcal penicillins and other agents has compromised therapy. Methicillin resistance was detected in *Staphylococcus aureus* shortly after the agent was introduced clinically and now there is a global epidemic of methicillin resistant *Staphylococcus aureus* (David & Daum, 2010). Strains sensitive to methicillin are classified as methicillin-sensitive *Staphylococcus aureus*, or MSSA.

MRSA is a serious current healthcare concern. MRSA was first detected in patients in hospitals and other health facilities, especially among elderly, debilitated and those require long term inmates' (Chambers, 2001). It was also found in patients who undergo surgery and other invasive procedures. In these settings, MRSA is referred to as health care-
associated MRSA (HA-MRSA). MRSA also has been found to cause infections in the community outside of hospitals and other health facilities and is known as community-associated MRSA (CA-MRSA) (Huang, et al., 2006). Both CA-MRSA and HA-MRSA are growing threats to the immune compromised individuals as well as to the general public.

Many of the MRSA isolates are becoming multi drug resistant and they are susceptible only to the glycopeptide antibiotics such as vancomycin. Even to vancomycin a low level resistance is emerging (Dhand & Sakoulas, 2012). A prolonged hospital stay or the indiscriminate uses of antibiotics are some of the common factors of the MRSA infections globally. The community prevalence of MRSA is increasing largely due to community associated MRSA strains (Salgado, et al., 2003).

*S. aureus* colonization can be an indication of a higher risk for subsequent infections, including MRSA. However, no community based prevalence study has been conducted to measure *S. aureus* carriage and reliable published data are lacking within Sri Lanka. The aim of this study was to describe the prevalence of Methicillin Sensitive *Staphylococcus aureus* (MSSA) and MRSA nasal colonization in the community and antibiotic susceptibility patterns of these isolates and their risk factors.

### II. MATERIALS AND METHODS

**Survey design and Collection of data.** This study was a community based descriptive cross sectional study. This study was conducted in two selected communities in Ratmalana divisional secretariat area. This area is within the Medical Officer of Health (MOH) – Dehiwela area.

**Study population.** Study population was 317, adults and children ≥ 2 years of age living in the two communities.

Sampling for the survey was done by obtaining nasal swabs. *S. aureus* screening was done for all participants. Known risk factors of MRSA carriage (defined as stay either in an acute care facility or a long term health care facility during last 3 months or 6 months before participation) (CDC, 2010) and other factors including residence with a patient with chronic disease, age, gender, occupation, number in household, education level, antibiotic use within 3 months or 6 months, residence with a patient who had been recently admitted to the hospital were examined.

**Laboratory Methods.** Nasal samples were collected from both anterior nares by using sterile culture swabs. Culture swabs were plated on Mac Conkey agar and incubated overnight at 35°C. Colonies with distinctive morphology of *S. aureus* were identified by routine laboratory methods and antimicrobial susceptibility tests were done by following the CLSI guidelines.

*S. aureus* isolates were screened for cefoxitin(30µg). Zone diameters were measured and recorded after 24 hours incubation at 35°C. Isolates determined to be resistant to cefoxitin were selected as MRSA and sensitive to cefoxitin were selected as MSSA (CLSI guidelines, 2012).

Inducible Clindamycin resistance were performed according to the CLSI method.

### III. RESULTS

#### Population characteristics.** Three hundred seventeen subjects ≥ 2 years of age were selected from the two communities. 62.77% were female and 37.22% were male. Among the age group selected, highest number of participants was included in the age group of 2-10 years (18.61%). When considering the education level, most of the participants had at least secondary education (52.68%). Both the communities were semiurban communities. 116 families were included for the study.

*S. aureus* carriage. Out of 317 subjects, 88 (27.76%) were identified as colonized with *S. aureus* (Table 1). *S. aureus* colonization was highest (26.13%) in the age group of 2-10 years and it was more frequent in households with more than 05 members. Hospital admissions and residences with a patient with a chronic disease were reported only 1.57% and 0.63% respectively for the *S. aureus* isolates. The most number of *S. aureus* carriage rate of 85.22%(75/88) was reported in the families with an income of Rs.20, 000/= and below, per month.

**MRSA carriage.** MRSA prevalence was 5.67% (18/317). MRSA prevalence was highest in the age group of 2-20 (55.54%). The most number of MRSA
carriage rate of 83.33% (15/18) was reported in the families with an income of Rs. 20,000/= and below, per month.

**Antibiotic Susceptibility testing.** Antibiotic susceptibility tests were done for the antibiotics Erythromycin, Clindamycin, Cefoxitin, Linezolid, Ciprofloxacin, Trimethoprim-sulfamethoxazole, Tetracycline and Gentamicin. High sensitivity was noted in Linezolid (100%), Ciprofloxacin (94.44%), Trimethoprim-sulfamethoxazole (94.44%), Tetracycline (77.77%) and Gentamicin (94.44%) for MRSA isolates (Table 3).

MSSA isolates were sensitive to Erythromycin, Clindamycin, Cefoxitin, Linezolid, Ciprofloxacin, Trimethoprim-sulfamethoxazole, Tetracycline and Gentamicin were 44.28%, 78.57%, 100%, 100%, 72.85%, 95.71%, 84.28%, 81.42% respectively (Table 2).

**Inducible Clindamycin resistance.** Inducible Clindamycin resistance was reported 7.14%, 44.44% among MSSA and MRSA respectively.

### Table 1: Prevalence of MSSA and MRSA

<table>
<thead>
<tr>
<th>Sample size</th>
<th>Total No. (%)</th>
<th>No. (%) of subjects with MSSA</th>
<th>No. (%) of subjects with MRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Study population)</td>
<td>317</td>
<td>88 (27.76)</td>
<td>18 (5.67)</td>
</tr>
</tbody>
</table>

Note:

SA - *Staphylococcus aureus*

MSSA-Methicillin Sensitive *Staphylococcus aureus*

MRSA- Methicillin Resistant *Staphylococcus aureus*

### Table 2: MSSA antibiotic susceptibilities in 70 samples

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>No. (%) of samples, by susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td><em>Cloxacillin</em></td>
<td>70(100.00)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>31(44.28)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>55(78.57)</td>
</tr>
<tr>
<td>Trimethoprim-</td>
<td>67(95.71)</td>
</tr>
<tr>
<td>sulfamethoxazole</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>51(72.85)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>59(84.28)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>57(81.42)</td>
</tr>
</tbody>
</table>

* sensitivity tested with cefoxitin (30 µg) disc

### Table 3: MRSA antibiotic susceptibilities in 18 samples

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>No. (%) of samples, by susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td><em>Cloxacillin</em></td>
<td>18(100.00)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>1(5.55)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>6(33.33)</td>
</tr>
<tr>
<td>Trimethoprim-</td>
<td>17(94.44)</td>
</tr>
<tr>
<td>sulfamethoxazole</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>17(94.44)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>14(77.77)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>17(94.44)</td>
</tr>
<tr>
<td>Linezolid</td>
<td>18(100.00)</td>
</tr>
</tbody>
</table>

* sensitivity tested with cefoxitin (30 µg) disc

**IV. DISCUSSION**

This was a community based study on MRSA and published data on MRSA within the Sri Lankan community is lacking. Overall, this study demonstrates the prevalence of *S. aureus* in the community and also the prevalence of community acquired methicillin resistant *S. aureus*. This study included young and adult participants of varied age groups from two semi urban communities in the western province.

We found that 27.76% (88/317) of the participants were colonized with *S. aureus*. MRSA prevalence of this study was 5.36% (18/317). Similar finding was noted in the studies of Alvarez & Reddy (2012) in the Indian communities which showed a MRSA prevalence of 4.6%-10.6%.

The risk factors for hospital associated MRSA colonization have been well described, the same has not fully described for CA-MRSA. In this study, we found crowding may contribute to *S. aureus* and MRSA colonization and infection.

In this study, nearly one fourth of the two communities had *S. aureus* colonization and Peak prevalence was found in 2-10 years age group.

In this study has shown that known risk factors for HA-MRSA such as hospital admission (0.315%), residence with a patient with chronic disease (0.315%) and residence with a patient who recently admitted to the hospital (0.63%) had no significant
association with MRSA carriage. Most of the antibiotics tested were sensitive (more than 75%), except for Erythromycin and Clindamycin. Antibiotic sensitivity pattern indicates, all the MRSA isolates were likely to be community acquired MRSA. However, further molecular studies are needed to confirm these findings.

REFERENCES


BIOGRAPHY OF AUTHORS

1 Author is a technical officer at Faculty of Medicine, General Sir John Kotelawala Defence University, Sri Lanka. Her research interests are community associated infection and antibiotic resistance.

2 Author is a senior lecturer and specialist in Medical Microbiology of Faculty of Medicine, General Sir John Kotelawala Defence University, Sri Lanka. His research interests are Hospital associated infection, infection control and antibiotic resistance.

3 Author is a senior lecturer of the Faculty of Medicine, KDU and a senior consultant community physician. His research interests are elderly, health promotion and NCD epidemiology. He is a Lieutenant Colonel of the Medical corps as a volunteer officer.

4 Author is a senior lecturer attached to the Department of Microbiology, Faculty of Science, University of Kelaniya. He is currently the Head of Department. His research interest include the construction of bacterial biosensors for population monitoring.
Evaluation of Random Donor Platelets Produced from Buffy Coat Stored for 24 hrs at Ambient Temperature. A Better Alternative to 8 hr Limitation

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Abstract:
Introduction-Whole blood derived platelets are made from platelet rich plasma (PRP) method or buffy coat method. In India majority of random donor platelets are prepared by PRP method. However, Buffy coat method offers the advantage of less platelet activation and fewer WBC contamination. Presently in India RDPs are prepared within 8 hour of whole blood collection, whereas, in Europe this time limit is up to 24 hours.

Aim-Our aim was to evaluate the platelet count, WBC contamination, platelet CD 62 P expression, and biochemical parameters of RDPs prepared from buffy coat within 8 hours and within 24 hours of collection.

Materials and method-We prepared 40 units of RDP by the buffy coat method from whole blood stored at room temperature within 8 hours of collection (Fresh BC),& another 40 units from buffy coat stored at 22°C for less than 24 hours (Stored BC). We analyzed the platelet counts, CD62P expression, WBC counts, glucose levels, pH, PO₂, PCO₂ in both the groups of RDPs, 24 hours after respective preparation.

Results-The platelet count from stored BC was higher in fresh BC. CD 62P expression was low in stored BC compared to fresh BC. There were no differences pH, pO₂, pCO₂ and glucose levels between fresh BC and stored BC. WBC contamination was more in fresh BC.

Conclusion-Our study stored BC contained higher platelet counts, less WBC contamination and less platelet activation. We conclude that RDP prepared from stored BC is the better method for RDP production

Keywords: Buffy coat, Platelet count, WBC contamination, Platelet activation, CD 62P expression.

I. INTRODUCTION

Buffy coat (BC) preparation of platelet is the most popular method of preparation of Random Donor Platelets (RDP) in countries such as Canada, UK and Europe. In Europe, there is an approximately 50:50 split between the use of BC- and apheresis-derived platelet concentrates (PC). [1] In Canada (Quebec exempted), 70% of platelets are derived from whole blood donation. Denmark, Finland, and the Netherlands prepare 85% to 95% of their concentrates by the BC method [2]. These countries demonstrate that a national platelet supply can be derived almost entirely from collected whole blood rather than relying upon apheresis PC production. [1, 2] A special kind of bag which has top and bottom outlets are used to prepare the platelet concentrate in this technique. Whole blood collected into the main bag is subjected to heavy spin and semi-automated expresser is used to expel out platelet poor plasma from the top port and red blood cells from the bottom port. Thirty mL of RBC and thirty mL of plasma remains in the main bag with majority of platelets and WBCs. The main bag is subjected to re-centrifuge in soft spin and platelet rich plasma is expelled out and collected from the top port, leaving buffy coat and red blood cells in the main bag.

In the United Kingdom and continental Europe, four to six buffy coats are combined, re-suspended in plasma from a male donor or in a platelet additive solution, and soft centrifuged to remove red cells and white cells. [3] Platelets can be prepared using this method from WB stored at
room temperature (not less than 20°C) for up to 24 hours [4]. Advantages of the buffy-coat method are that there is less activation of platelets than in the PRP method because in the buffy-coat method, platelets are cushioned against red cells during the hard spin. Plasma units prepared from buffy-coat-depleted units have approximately 41 mL more plasma than plasma units prepared by the PRP method. Platelets prepared by the PRP method result in 21% of the plasma and 19% of the platelets remaining with the red cells. Therefore, the hematocrit of the packed RBCs produced with the PRP method is lower (51%) than in the buffy-coat method (60%). Buffy coat depletion also results in a 13% loss of the donated red cells.[5] In PRP method of platelet preparation PRP is subjected to hard spin leading to formation of platelet pellet. Pelleting make close contact of platelets which make them activate temporarily. In contrast buffy-coat method gives the luxury of cushion to platelets among RBC during the hard spin. Therefore platelets are less activated than PRP method. [6]

Platelet activation during processing and throughout storage is accompanied by surface expression of sequestered granular membrane proteins (P-selectin and CD63) and conformational changes of the fibrinogen receptor, GP-IIb/IIIa. [7] P-selectin (also known as CD62P, GMP-140, and PADGEM) is an integral membrane protein found in α-granules that becomes expressed on the surface of activated platelets after granule release. [8]

In India when RDP is prepared by the buffy coat method, it is done so, almost entirely from the whole blood stored at room temperature, for less than 8 hrs as per DGHS guidelines. [9]

The aim of our study was to evaluate the platelet count, WBC contamination, platelet activation and other biochemical parameters of platelet concentrate prepared from buffy coat within 8 hrs and within 24 hrs of collection, respectively, in order to assess and compare the two methods.

II. MATERIALS AND METHOD

Forty units of RDP prepared from fresh buffy coat separated from whole blood within 8 hrs of collection (Fresh BC) and forty units of RDP prepared from buffy coat stored at 22°C, overnight () were studied in an unpaired study design.

Eighty units of whole blood were collected in to quadruple bag (Fenwal, Lake Zurich, IL 60047, United States) and subjected to high spin separation within 8 hrs of collection. Semi-automated plasma expresser (Optipress – Fenwal, Lake Zurich, IL 60047, United States) was used to express out the platelet poor plasma from the top port of the bag and red blood cells were expressed out from the bottom port leaving buffy coat and platelets in the primary collection bag. Out of them 40 units were subjected to rest for one hour and thensubjected to soft spin centrifugation. Platelets were expressed out from the top port using semi-automated plasma expresser, leaving the buffy coat and RBC in the primary collection bag. The other 40 units were left undisturbed overnight at 22°C and RDPs were prepared after soft spin centrifugation on the following day. Samples were taken after 24 hrs of preparation of platelets and day 5 of blood collection in both the groups.

All in vitro assays were undertaken according to the established validated method. Platelet counts and WBC counts were determined by using automated cell counter (Sysmax, Wakinohama-Kaigandori, Chuo-ku, Kobe, 651-0073, Japan) in the blood bank. In vitro glucose values were determined by the biochemistry laboratory of the hospital. [National accreditation board of laboratories (NABL) accredited lab]. pO2, pCO2 and PH were determined by using blood gas analyzer (Roshe, Carolina Inc.Old Marion Highway, Florence, SC, United States). Platelet surface CD 62P expression were measured by using flow cytometry (FACS Calibur, BD Biosciences, Qume Drive,San Jose, California, USA). Two samples from each platelet units (50 uL) were taken and one sample was stained by 10 uL monoclonal mouse antibodies and phycoerythrin according to the manufacture’s instruction. Second sample was analyzed without staining. First unstained sample was analyzed, followed by the stained sample. 6,000 events per second were counted in both samples by the flowcytometer. Result graphs of both samples were superimposed and results were obtained as a percentage of activation.

Statistical analysis were done by using IBM SPSS statistic software version 21 and p < -0.05 were considered as statistically significant.
Randomly selected platelet samples were sent for bacteriological culture to the microbiology department of the hospital.

III. OBJECTIVES

Our primary objective was platelet count in the RDP and secondary objectives were WBC count, pH, glucose, pO2, pCO2 and platelet surface CD 62P expression.

IV. RESULTS

At 24 hrs after preparation:

Fresh BC and Stored BC showed mean platelet counts of $5.7 \times 10^{10} \pm 1.57$ and $6.32 \times 10^{10} \pm 1.18$ per unit, respectively. Among the fresh BC, 31 (77.5%) out of 40 units contained more than $6 \times 10^{10}$ platelets per unit whereas in stored BC only 36 (90%) units out of 40 units contained more than $6 \times 10^{10}$ platelets per unit. Mean WBC contamination in fresh BC was $5.88 \times 10^6 \pm 2.85$ and stored BC was $5.55 \times 10^6 \pm 1.53$ per unit respectively. (Fig 1) Fresh BC, 31 units and stored BC 35 units out of 40 units each, contained less than $5.5 \times 10^6$ WBC respectively. Mean CD 62P expression in fresh BC and stored BC showed 33.35% ± 13.75 and 19.5% ± 14.45 respectively (Fig 2). Range CD 62P expression of platelet in fresh BC was 14% to 55% and in stored BC, it was 4% to 44%. Mean plasma glucose levels in fresh BC was 389 mmol/L ± 67.91 and stored BC was 360 mmol/L ± 62.18. Mean pH of the fresh BC was 6.99 ± 0.23 whereas in stored BC was 6.91 ± 0.21. Mean pCO2 of both fresh BC and stored BC were 8.55 kPa ± 2.72 and 8.4 ± 2.58 respectively. Mean pO2 of the fresh BC was 16.6 kPa ± 3.46 and stored BC 15.37 kPa ± 2.82. Microbiological cultures of randomly selected samples of both fresh BC and stored BC were found to be sterile. (Table 2)

Table 1: 1 Mean values of each variables of platelet concentrate of 24hrs after of preparation.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Fresh BC</th>
<th>Stored BC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets x 10^{10} per unit</td>
<td>5.7 ± 1.57</td>
<td>6.32 ± 1.18</td>
</tr>
<tr>
<td>WBC x 10^6 per unit</td>
<td>5.88 ± 2.85</td>
<td>5.55 ± 1.53</td>
</tr>
<tr>
<td>CD 62P expression %</td>
<td>33.35 ± 13.75</td>
<td>19.5 ± 14.45</td>
</tr>
<tr>
<td>Glucose mmol/L</td>
<td>389 ± 67.91</td>
<td>360 ± 62.18</td>
</tr>
<tr>
<td>pO2 (kPa)</td>
<td>17.12 ± 4.1</td>
<td>16.5 ± 3.12</td>
</tr>
<tr>
<td>pCO2 (kPa)</td>
<td>7.7 ± 3.07</td>
<td>7.7 ± 3.07</td>
</tr>
<tr>
<td>pH</td>
<td>6.99 ± 0.23</td>
<td>6.91 ± 0.21</td>
</tr>
</tbody>
</table>

At 5th day of storage:

Fresh BC and Stored BC showed that mean platelet counts at the 5th day of storage were $5.5 \times 10^{10} \pm 1.4$ and $5.83 \times 10^{10} \pm 1.05$ per unit, respectively. Mean WBC contamination in fresh BC after 5 days of storage was $5.33 \times 10^6 \pm 2.69$ and stored BC was $4.93 \times 10^6 \pm 0.59$ per unit respectively. CD 62 P expression in fresh BC and stored BC at the end of the 5 day storage showed that 38.47% ± 13.18 and 22.3% ± 13.86 respectively. Mean plasma glucose levels in fresh BC was 379.5 mmol/L ± 56.48 and stored BC was 348.35 mmol/L ± 53.13. Mean pH of the fresh BC was 6.93 ± 0.27 where as in stored BC was 6.85 ± 0.25. Mean pCO2 of fresh BC and stored BC were 16.5 kPa ± 3.46 and 15.37 kPa ± 2.82 respectively. Microbiological cultures of fresh BC and stored BC were found to be sterile. (Table 2)

Table 2: 1 Mean values of each variables of platelet concentrate of 5 days after of preparation

<table>
<thead>
<tr>
<th>Variables</th>
<th>Fresh BC</th>
<th>Stored BC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets x 10^{10} per unit</td>
<td>5.5 ± 1.4</td>
<td>5.83 ± 1.05</td>
</tr>
<tr>
<td>WBC x 10^6 per unit</td>
<td>5.33 ± 2.69</td>
<td>4.93 ± 0.59</td>
</tr>
<tr>
<td>CD 62P expression %</td>
<td>38.47 ± 13.18</td>
<td>22.3 ± 13.68</td>
</tr>
<tr>
<td>Glucose mmol/L</td>
<td>379.5 ± 56.48</td>
<td>348.35 ± 53.13</td>
</tr>
<tr>
<td>pO2 (kPa)</td>
<td>16.6 ± 3.46</td>
<td>15.37 ± 2.82</td>
</tr>
<tr>
<td>pCO2 (kPa)</td>
<td>8.55 ± 2.72</td>
<td>8.4 ± 2.58</td>
</tr>
<tr>
<td>pH</td>
<td>6.93 ± 0.27</td>
<td>6.85 ± 0.25</td>
</tr>
</tbody>
</table>

V. DISCUSSION

In our study we studied the effect of ambient temperature on platelet concentrate which was prepared from overnight hold buffy coat, and results were compared with platelet concentrate prepared from fresh buffy coat within 8 hrs of collection. It was found that the stored BC contained comparatively higher platelet counts, and were less activated compared to the fresh buffy coat prepared platelet concentrate.
Lower platelet count in the Fresh BC may be due to relatively less resting period of buffy coat before preparation of platelets. Platelets within the 8 hrs of collection, are still in a relative stage of aggregation and activation due to the centrifugation they were subjected to, while being prepared. Due to these possibilities platelets were trapped among the concentrated WBCs and could not come to the plasma layer during soft centrifugation. On the other hand stored BC, have the advantage of relatively more resting period during which period the aggregation and activation of platelets would reduce, which will eventually lead to more and more platelet release into the plasma layer in soft second centrifugation of platelet production. In our study 10.87% more platelets were concentrated in stored BC compared to fresh BC. Margriet J, Dijkstra-Tiekstra, Willeke Kuipers, at al explained in their study that the overnight platelet concentrate (PC) showed higher PLT count (approx. 460 × 10⁹/PC vs. approx. 310 × 10⁹/PC) compared to that of fresh buffy coat derived PC.[10]. This finding was comparable with our results.

Mean WBC contamination of fresh BC and stored BC was 5.88 ± 2.85 and 5.55 ± 1.53 WBC x 10⁶ per unit respectively. There was more contamination with WBC (5.94%) more WBC s in fresh BC compared to stored BC. Overnight exposure to room temperature (20 – 24°C) lead to lysis of the WBC s, and, overnight undisturbed standing may have settled down the WBCs in the stored buffy coat. Therefore stored BC contained less WBC s compared to fresh BC. Fig 1 CD62 P expression in the 24 hrs after the production, was 33.35 ± 13.74% in fresh BC and 19.5 ± 14.44 % in stored BC respectively. Thus in fresh BC, 13.85% more platelets were activated, compared to that of stored BC. However this difference was not statistically significant. At the end of the 5th day of storage both fresh and stored BC platelets expressed CD 62 P more than their respective 1st day values. In fresh BC 5.13% more platelets expressed CD 62 P after 5 days of storage when compared to that of fresh BC in 1st day. This difference was statistically significant. In stored BC 2.8 % more platelets were activated when 1st day and 5th day were compare but was not statistically significant. Comparison of both fresh and stored BC at the 5th day of storage has shown that fresh BC contained 11.18 % CD 62 P expressed platelets than in the stored BC.(Table 2) High expression of CD 62 P of platelets in fresh BC appear to be due to shorter resting period compared to stored BC. Longer resting period would result in disaggregation as well as less activation of the platelets. However the platelets expressed CD 62 P more and more during the storage due to reduced pH.

![WBC contamination of fresh and stored BC in 1st and 5th day](image)

**Figure 1:**

We analyzed metabolic parameters of the platelets, such as glucose pO₂, pCO₂ and pH after 24 hrs and 5th day of storage. Amount of glucose (mmol/L) in fresh BC was 389 ± 67.91 and in stored BC was 360 ± 62.18. WBC s and platelets stored overnight in room temperature, metabolized glucose and produced lactic acid as a byproduct. This was the reason of reduced amount of glucose in stored BC. Due to increased amount of lactic acid production in stored BC, pH has reduced to 6.91 compared to fresh BC (6.99). However pH was within the quality control parameters of buffy coat platelets. The pO₂ and pCO₂ values of both products were almost similar. However none of the metabolic parameters between two groups were statistically significant even after 5 days of storage. Margriet J, Dijkstra-Tiekstra, Willeke Kuipers, at al found that the overnight PC higher pCO₂, and lactate concentration and lower pH, pO₂, glucose concentration, CD62P expression (until Day 5). We did not measure the lactate concentration but rests of the findings are comparable with our results [10]. In another study M.J. Dijkstra-Tiekstra, P.F. van der Meer, R. Cardigan, at el studied the lactate, pCO₂, and hypotonic shock response pH, glucose, pO₂, and CD62P expression swirling effect, white blood cell count, annexin V binding, and aggregation between overnight and fresh buffycoat derived PC. They found significant difference in lactate, pCO₂, hypotonic shock response, pH, glucose, pO₂, and CD62P expression between the fresh and overnight held PC. In our study we found
that findings were not statistically significant [11]. Another similar study done by Fa Qiang Lu, Wei Kang, Yu Peng, Wei Ming Wang revealed that the platelet yield in PCs prepared from an overnight-hold WB sample higher, while CD62P expression and annexin V binding were lower (p < 0.05). These findings were comparable with our results [12].

Platelets experience a progressive decline in function accompanied by characteristic morphologic changes. Studies document up to 20% loss of platelet recovery through 5 days of storage. In our study 12.75% loss of platelet was observed in fresh BC at the 5th day of storage, whereas the amount lost in the stored BC at the end of storage was 6.82%. [8] Activation of platelets was the main reason for platelet loss during storage. Less activated platelet in stored BC showed a much lesser loss during storage in our study; 15% in stored BC versus 30% after 5 days of storage. Exposure to room temperature for longer time in stored BC resulted in lysis of WBC more than in fresh BC. This has an additional advantage of less WBC contamination in stored BC. However the effects of cytokines, released in to the platelet concentrate due to lysis of WBC has to be studied further. An added advantage of stored BC is that when WBC s are in contact with prospective bacterial contaminants for a longer period of time, as is the case with stored BC, then the chance of increased pathogen destruction due to phagocytosis is higher.[13].

However keeping buffy coat for 24 hrs before preparation of platelets, reduce the storage time of platelets for 24 hrs is a drawback of the study.

VI. CONCLUSION

Our study concluded that stored BC contained higher platelet counts, less WBC contamination and less platelet activation. Platelets can be prepared from stored BC, during business hours of following day. This would provide for better supervision, ensuring a better product. This would benefit blood banks where the preparation of components are delayed due to longer transportation time either from satellite blood banks or blood donation camps, which is a common problem in India. Also fewer RDPs are discarded due to low platelet counts, as this method recovers comparatively more platelets. Also less number of units are required to be transfused to patients, since the quality per unit is better, which eventually leads to patient exposure to fewer donors. We conclude that RDP prepared from buffy coat method stored at room temperature for 24 hrs, is a better method for production of platelet concentrate.

<table>
<thead>
<tr>
<th></th>
<th>Fresh BC</th>
<th>Stored BC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1 (%)</td>
<td>33.35 ±13.74</td>
<td>19.5 ±14.44</td>
</tr>
<tr>
<td>Day 5 (%)</td>
<td>38.48 ±13.18</td>
<td>22.3 ±13.86</td>
</tr>
</tbody>
</table>

Table 2: CD 62 P expression (%) in fresh BC and stored BC in day 1 and day 5 of storage.

REFERENCES


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BIOGRAPHY OF AUTHORS

1Author is a Transfusion medicine specialist in Army Hospital Colombo. He has published articles in International journals in blood banking and immunohaematology.

2 Author is a post graduate student of Transfusion medicine and immunohaematology in Armed forces medical college, Pune Maharatra, India.
Assessing the Validity of the Threefold Conversion between Hemoglobin and Hematocrit for the Determination of Anemia in Pregnancy

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Abstract –

Introduction: The worldwide prevalence of anemia is higher among pregnant women. Anemic status is determined by the measurement of Hemoglobin (Hb) or Hematocrit (Hct). Hct (%) is usually defined as three times the value of Hb (g/dl). But the crude relationship between the two measures may be modified due to several factors such as; age, sex, season of survey and disease conditions. This study was therefore undertaken with the intent of assessing the validity of the 3-fold conversion between Hb and Hct to assess anemia in pregnant women.

Method: The Hb concentrations and Hct values from 70 pregnant mothers, aged 18-30 years, in their first trimester of pregnancy, from the antenatal clinics of Teaching Hospital, Mahamodara were analyzed. The relationship between the concurrent measures of Hb and Hct was defined by linear regression analysis and the validity of the 3-fold conversion was assessed.

Results: The prevalence of anemia as defined by both Hb and Hct levels was 17%. Almost 59% of the microhematocrit values wrongly estimated Hb using 3-fold conversion. Sensitivity and specificity results obtained were below the reliability of clinical measurement. Regression models show that the association between the cut-offs of Hb and Hct is not dependent only on a simple conversion factor and correlation coefficient results are inadequate to conclude that there is a significant relationship between the two variables. A significant difference was observed between the values obtained using the two methods (t= 7.182, p <0.001).

Conclusions: The relationship between Hb and Hct is not exactly 3 in pregnant women and there is no simple conversion factor between the two measures. Since the Drabkin’s method still has the advantage of being an international standard, this study argues for the consistent use of Hb rather than Hct in the assessment of anemia in pregnancy.

Keywords – Hemoglobin, Hematocrit, Pregnancy, three-fold conversion

I. INTRODUCTION

A. Anemia in pregnancy

Anemia is a condition in which there is decreased level of Hb than normal or there is decreased number of RBC’s than the normal value (Beutler & Waalen, 2006). It is a common and severe problem in many developing countries. Anemia in pregnancy is a special issue where, the normal physiological increase of both plasma volume and red cell mass with an excessive increase in plasma volume causes hemodilution in a pregnant woman resulting in a reduction of Hb levels approximately up to 11g/dl (Blackwell, 2008). According to the CDC (Centers for Disease Control and Prevention) criteria Hb cut-off used to define anemia during the first trimester of pregnancy is 11 g/dl and the corresponding cut-off for Hct is 0.33 (Gjorup, 1986).

Anemia in pregnancy is associated with deleterious effects such as fetal cerebral vasodilatation, increased risk of prematurity, spontaneous abortion, low birth weight, fetal death as well as increased risk of maternal mortality (Kalaivani, 2009). Pregnant women with anemia have a mortality rate three to five times higher than normal and a stillbirth rate six times higher than normal (Kalaivani, 2009). According to the United Nations estimates, approximately half of pregnant women suffer from anemia worldwide while the prevalence of anemia during pregnancy is 75% in South Asia (Karaoglu et al., 2010; Brabin et al,
Since early detection and effective management of anemia in pregnancy contributes substantially to the reduction in maternal mortality and stillbirth rate, it is crucial that all pregnant women are screened for anemia during pregnancy (Kalaivani, 2009; Den Broek et al., 1999; Sherard & Newton, 2001).

B. Screening for anemia during pregnancy
Anemia is typically determined by measuring the Hb concentration in blood. In developed countries, where the prevalence of anemia is below 20%, an accepted standard practice is that all women have at least one measurement of Hb during the course of pregnancy (Brabin et al., 2001). However, Packed Cell Volume (PCV) or Hct has been widely used as an alternative to Hb in various antenatal clinics and peripheral units.

C. Hemoglobin and Hematocrit
Both Hb and Hct refer to specific characteristics of red blood cells, however, they measure different factors. Hb provides a direct measure of the Oxygen carrying capacity of the blood, whereas Hct provides an indirect measurement of this. Both parameters can be assessed either with an automated blood counter or by manual methods such as microhematocrit method for Hct or colorimetric method for Hb (Sherard & Newton, 2001). Both Hct and Hb levels could be affected by factors such as the method and equipment used for its multivariate model: Hct = 3x Hb (De Benoist et al., 1993).

Hb is considered to be superior to Hct for the purpose of monitoring anemia because of the availability of international reference standard preparations (Quinto et al., 2006). In resource poor settings where automated hematology analyzers are not available, the Cyanmethemoglobin method is often used for Hb estimation. Nevertheless this method is time consuming and its disposal may create a problem due to large volumes of reagent which contains cyanide constitute a potential biotoxic hazard (Kelleher et al., 2001).

In situations where limited resources are available and the technical support is poor, a simple screening tool is likely to perform better than sophisticated methods that depend on correct dilutions and preparation of standards (Brabin et al., 2001). Measurement of Hct using microhematocrit method is one such laboratory investigation which is simple to use, cheap and provides results within a very short period of time (5 min). As per the literature, microhematocrit method has an adequate level of accuracy and precision for clinical utility and therefore in many settings where automated methods for Hb determinations are not available, Hb values are estimated using observed Hct levels (Chakravarthy et al., 2012). In general, in a rural setting, the running costs for Hct are very low and therefore in studies involving large populations it is cheaper to measure Hct (WHO, 2000). Further it is a less hazardous method that can be performed with less qualified personnel. Even a finger prick capillary blood sample is sufficient for performance of Hct testing by microhematocrit method.

D. Three-fold conversion between Hb and Hct
It is generally assumed that the conversion from Hb to Hct is pretty straightforward. Using the Hct value, there is a rough conversion factor of 3 which converts the Hct value to approximate Hb level. For example, if 33% of the blood contains red blood cells by volume, the Hb content would be about 33/3 = 11 g/dl (Jordan, 2009).

E. Factors affecting the 3-fold conversion
The crude relationship between Hb and Hct levels may be modified due to several factors. The literature has highlighted the fact that, it may vary with age, sex, season of survey and disease conditions such as malaria (De Benoist et al., 1993). The relationship between Hct and Hb is expressed with the Mean Corpuscular Hemoglobin Concentration (MCHC). The MCHC varies depending on the type of anemia. An increased MCHC is seen in spherocytosis, whereas decreased levels may indicate iron deficiency, blood loss or thalassemia. It could be the case that obtaining a single conversion factor is not feasible, as the relationship depends on the prevalence of anemia in each population and on the type of anemia as well (De Benoist et al., 1993).

The retrospective data of the studies done on the matter in different laboratories in different countries shows that the assessment of anemia using the 3-fold conversion between Hb and Hct has become a debatable issue with some studies showing positive correlation and some showing absence of any correlation (Carneiro & Drakeley, 2007; Lee et al., 2008; Rycel et al., 2009). Therefore the potential for further improvements...
in the conversion factor certainly merits further investigation and analysis.

II. METHODS

A. Subjects and sampling

K₂EDTA blood samples were collected by nursing officers in the course of routine Hb estimation in pregnant mothers (n=70) on their first visit to the ante-natal clinics (both hospital clinic and university; obstetrics and gynecology clinics) at Teaching Hospital Mahamodara. The first visit is usually made at 9 – 12 weeks gestation. The inclusion criteria included; subjects in the first trimester of pregnancy, aged between 18-30 and no history of chronic illness. Exclusion criteria included; age younger than 18 and older than 30, previous history of nutritional or hemolytic anemia and a history of hemoglobinopathies. Written consent was obtained from each participant and an interviewer administered questionnaire was filled in. The subjects were selected using non-probability purposive sampling technique. The minimum sample size required in purposive sampling is often fewer than 30. To increase the reliability of the results, considering the time and resources provided, sampling was continued up to 70. It was the size of sample affordable with the two months’ time period of data collection. The study period was January 2013 – January 2014 (from literature review and proposal writing to dissemination of knowledge).

B. Laboratory methods

Blood samples were processed to measure Hb by Drabkin’s method (cyanmethemoglobin method) using UV-1800, SHIMADZU recording double beam spectrophotometer (USA). Hct was assessed by centrifugation using Universal Microhematocrit Centrifuge (Hettich instrument, Germany) according to standard microhematocrit procedures (12000g, 5min).

C. Statistical analysis

All mothers with Hb values less than 11 g/dl were categorized into anemic group. Anemic pregnant mothers were further classified as severely anemic (Hb < 8 g/dl), moderately anemic (Hb 8 – 9.9g/dl) and mildly anemic (10 – 10.9g/dl) (Gjorup, 1986). Descriptive analysis was done first to explain the variation in the variables studied. Linear regression models were evaluated in order to evaluate the relationship between Hb and the Hct values.

Sensitivity and specificity were calculated to assess the validity of the 3-fold conversion.

III. RESULTS

A. Prevalence of anemia among pregnant women

Approximately one sixth (17%) of the population had anemia during the first trimester of pregnancy evidenced by Hb concentrations of 8-11 g/dl. According to the results of both Drabkin’s method and Hct method, 14.3% of the pregnant mothers were classified as having mild anemia and 2.9% as having moderate anemia (Table 1). About 83% pregnant mothers with an Hb concentration > 11 g/dl were classified as non-anemic.

![Image](https://via.placeholder.com/150)

**Table 1. Distribution of anemia (n=70) in the study population**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Definition</th>
<th>Frequency, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>Hb 10 – 10.9 g/dl</td>
<td>10 (14.3)</td>
</tr>
<tr>
<td>Moderate</td>
<td>Hb 8 – 9.9 g/dl</td>
<td>2 (2.9)</td>
</tr>
<tr>
<td>Severe</td>
<td>Hb &lt; 8 g/dl</td>
<td>-</td>
</tr>
<tr>
<td>Total anemia</td>
<td>Hb &lt; 11 g/dl</td>
<td>12 (17)</td>
</tr>
</tbody>
</table>

Approximately 50% out of the mild anemic cases which were classified as mildly anemic by Hb (Drabkin’s method) were not considered anemic using the Hct level. For moderate anemia, the agreement was similar as 50% of the pregnant mothers classified as having moderate anemia by Hb did not classify as moderately anemic when using the Hct level. About 10% of subjects classified as mildly anemic by Drabkin’s method were considered as moderately anemic by the Hct method (Table 2).

![Image](https://via.placeholder.com/150)

**Table 2. Comparison of the results obtained by Drabkin’s method and Microhematocrit method**

<table>
<thead>
<tr>
<th>Hb values obtained by Drabkin’s method (g/dl)</th>
<th>(g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 – 9.9</td>
<td>9.9</td>
</tr>
<tr>
<td>10 – 10.9</td>
<td>10.9</td>
</tr>
<tr>
<td>11 – 11.9</td>
<td>11.9</td>
</tr>
<tr>
<td>12 – 12.9</td>
<td>12.9</td>
</tr>
<tr>
<td>13 – 13.9</td>
<td>13.9</td>
</tr>
<tr>
<td>≥ 14</td>
<td>Total</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hb values by Hct/3</th>
<th>1</th>
<th>1</th>
<th>0</th>
<th>0</th>
<th>0</th>
<th>0</th>
<th>0</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 – 9.9</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>10 – 10.9</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>11 – 11.9</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>29</td>
</tr>
<tr>
<td>12 – 12.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>13 – 13.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>≥ 14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>70</td>
</tr>
</tbody>
</table>

Total 2 10 16 25 13 4 70
Hb values obtained by Drabkin’s method (g/dl)

<table>
<thead>
<tr>
<th>Hb values by Hct/3 (g/dl)</th>
<th>8 – 9.9</th>
<th>10 – 10.9</th>
<th>11 – 11.9</th>
<th>12 – 12.9</th>
<th>13 – 13.9</th>
<th>≥14</th>
</tr>
</thead>
<tbody>
<tr>
<td>No: of samples</td>
<td>70</td>
<td>70</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Hb (g/dl)</td>
<td>11.6</td>
<td>12.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median Hb (g/dl)</td>
<td>11.7</td>
<td>12.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>0.81</td>
<td>1.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lowest Hb (g/dl)</td>
<td>9.70</td>
<td>9.40</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Highest Hb (g/dl)</td>
<td>13.3</td>
<td>15.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>3.60</td>
<td>5.70</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Student t-test</td>
<td>7.182</td>
<td>P&lt; 0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B. Drabkin’s method Vs. 3-fold conversion

A comparison of different variables for Drabkin’s method and 3-fold conversion by microhematocrit method is given in table 3.

Table 3. Comparison of Hct/3 measurements and Drabkin’s method for Hb estimation

<table>
<thead>
<tr>
<th>Hemoglobin concentrations (g/dl)</th>
<th>Results</th>
<th>True positive</th>
<th>True negative</th>
<th>False positive</th>
<th>False negative</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8 – 10</td>
<td>16</td>
<td>4</td>
<td>10</td>
<td>10</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>9.9</td>
<td>10.9</td>
<td>11.9</td>
<td>12.9</td>
<td>13.9</td>
<td>62.5</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>8 – 11</td>
<td>10</td>
<td>4</td>
<td>10</td>
<td>11</td>
<td>62.5</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>9 – 12</td>
<td>12</td>
<td>2</td>
<td>12</td>
<td>13</td>
<td>62.5</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>10 – 13</td>
<td>13</td>
<td>2</td>
<td>13</td>
<td>14</td>
<td>62.5</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>≥14</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>11</td>
<td>62.5</td>
<td>48</td>
</tr>
</tbody>
</table>

C. Validity of the 3-fold conversion between Hb and Hct

The sensitivity and specificity results of the 3-fold conversion considering the whole population were 58.3% and 91.4% respectively. The validity of the conversion factor at different cut-off points of Hb concentrations considering the Drabkin’s method as the Gold Standard is shown in Table 4.

Table 4. Validity of the 3-fold conversion between Hb and Hct at different cut-off points of Hb concentrations

D. Correlation

Figure 1, 2 and 3 shows the relationship between Hb and Hct levels for the study samples, using different regression models. A positive correlation was observed between the results of the two methods with a correlation coefficient (r) of 0.799 ($r^2 = 0.637$) considering the whole study population. Though the results showed good correlations there were some random differences in readings, especially at higher levels of Hb concentrations. The intercept (a = 13.91) and slope (b = 1.711) suggest that there is a bias in the results (ideally a = 0 and b = 3) and there is no simple conversion factor between the two measures as the relationship is:

$$Hct = (1.711 \times Hb) + 13.91$$ (Figure 1).

At mild anemia, the relationship between Hb and Hct as expressed by the regression line was: $Hct = (2.601 \times Hb) + 4.621$ with a 0.559 of Correlation Coefficient (r) (Figure 2). The number of subjects with moderate anemia (2 mothers) was inadequate to conclude with a relationship between the two values.

To avoid the possibility of bias, separate regressions were performed for the determination of the conversion factor for non-anemic pregnant women (Figure 3). The equation derived through linear regression for non-anemic pregnant mothers was $Hct = (1.644 \times Hb) + 14.78$ with a strong positive correlation (r=0.715) between the two variables.
DISCUSSION

Anemia in pregnancy constitutes a real concern all over the world including developing countries like Sri Lanka. A research done in 2001 revealed that the prevalence of anemia among Sri Lankan pregnant women was 29.3% (WHO, 2001). Findings of the present study (prevalence of 17%) were not close to those figures and the reduction in anemia prevalence is expectable due to the socio-economic growth of Sri Lankan population over the past few years since governments conducted programs related to mother and child health such as antenatal care, nutrition education of the public etc.

As indicated previously, anemia may have an adverse impact on maternal and fetal well-being and pregnancy outcome. Significant effort is therefore given to monitoring and responding to hematological parameters (Bland & Altman, 1986). The gold standard laboratory investigation for the detection of anemia is measurement of Hb concentration using Cyanmethemoglobin (Drabkin's) method. The conversion factor of 3 which converts the Hct value to approximate Hb level was considered in this study.

The mean Hb value obtained from Drabkin’s method was 12.2 g/dl while the corresponding value derived from 3-fold conversion of Hct was 11.6 g/dl. The lower mean value suggests that, the 3-fold conversion would underestimate the Hb values. The range of Hb results by Drabkin’s method was more elaborate (5.7 g/dl) while the results derived by Hct method was more compact (3.6 g/dl). This suggests that Drabkin’s method is more sensitive and has an obvious advantage over the 3-fold conversion which is less sensitive.

The results showed a consistent bias between the two measures, with Hb concentrations by Drabkin’s method being higher than the values derived from 3-fold conversion in 81% of the observations. There was a significant difference between the two measures especially at higher Hb concentrations and this was confirmed by the student t-test (p<0.001). The detection of anemia was correctly made in 50% and 40% of moderate and mild anemic cases respectively by Hct method. About 10% of subjects classified as mildly anemic by Drabkin’s method were considered as moderately anemic by the 3-fold conversion. These variations in Hb results by the two methods would compromise the validity of the 3-fold conversion.

For the purpose of screening an antenatal population for anemia, high sensitivity is desirable since it is important that as many individuals as possible with anemia have a positive test result. But the sensitivity results obtained from this study was below the reliability of clinical measurement for Hct conversion method. With the increase in Hb concentrations the sensitivity results seemed to be decreasing while the specificity results varied.

Previous studies have used correlation to compare two measurement methods (Bland & Altman, 1986). Regression models show that the association between the cut-offs of Hb and Hct was not dependent only on a simple conversion factor (Figure 1). Though a positive correlation ($r = 0.799$)
was observed when considering Hb and Hct values, the line of best fit indicates that only 64% \( (r^2 = 0.637) \) of the variation in Hct values is explained by the regression line. 36% of the variation may be due to other factors which are not captured in our regression model. As mentioned in the literature, the factors such as age, seasonal variations and exposure to disease conditions such as malaria may have modified the crude relationship between Hb and Hct (Bland & Altman, 1986).

When considering the non-anemic pregnant women, the correlation between the two variables was a strong positive correlation with a Correlation Coefficient of 0.715 (Figure 3). The Correlation Coefficient for mild anemia (Figure 2) was 0.559 \( (r^2 = 0.312) \) and those results were comparable to the research by Lee SJ et al (2008). However, as suggested by Cornbleet & Gochman (1979), in any analysis if the data is collected over a narrow range, the estimate of the regression parameters is relatively imprecise and may be biased. The correlation coefficient can be used as a guide to assess the adequacy of the comparative method range in overcoming this problem and the range of data can be considered adequate if \( r > 0.975 \). Considering the narrow range involved in this study, correlation coefficient results are inadequate to conclude that there is a significant relationship between the two variables.

It was possible to derive comparable Hb levels from Hct method using different equations at different cut-off concentrations in this study (see Figure 1-3). The requirement of different conversion factors would compromise the reliability of practical use of Hct in determination of Hb results. Therefore the standard 3-fold conversion between the two measures cannot be considered as valid for the assessment of anemia.

The reasons for these different conversion factors were not analyzed during this study but it is suspected that physiological changes during pregnancy may have an effect on it. Errors of microhematocrit procedure such as sampling errors (prolong stasis, inadequate mixing with anticoagulants), errors in filling or sealing, reading errors and packing errors during the microhematocrit procedure may also have given rise to the difference between the results of two methods. Also there is a possibility of environmental factors and subject’s differences affecting the results due to the long interval between sampling and performing the test. There can be time dependent shrinkage of red cells which can contribute to Hct results. However the anticoagulant used was K$_3$-EDTA which has shown least influence on analyze on storage (CLSI guidelines).

V. CONCLUSIONS

These data show that Hb levels cannot be derived from the Hct values with an acceptable accuracy using the general rule of dividing by 3. The conversion factor between Hb and Hct is not exactly 3 in pregnant women and there is no simple conversion factor between the two measures. Since the Drabkin’s method still has the advantage of being an international standard, this study argues for the consistent use of Hb rather than Hct in the assessment of anemia in pregnancy.

VI. LIMITATIONS

Exclusion of pregnant mothers from other antenatal clinics (MOH clinics) and hospitals may limit the specificity of this investigation. Not performing serum folate level, peripheral blood film microscopy and serum iron profile (for the confirmation and differentiation of anemia) due to financial constraints was another shortcoming. ROC curve to assess validity of the 3-fold conversion was not applicable due to small sample size.

ACKNOWLEDGEMENT

Authors are grateful to the nursing and laboratory staff of the Teaching Hospital Mahamodara, Galle, for their kind collaboration at different stages of the study. The enormous support given by the staff of the Medical Laboratory Sciences Degree Program, Faculty of Medicine, University of Ruhuna is also acknowledged.

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Outcome of Pregnancy Complicated by Infective Endocarditis: A Review of Published Literature over Past Three Decades

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Abstract

Background - Infective endocarditis (IE) is a rare complication during pregnancy but it associates with high maternal and foetal mortality. Our aim was to study maternal and foetal outcomes in published cases of IE during pregnancy.

Method – A PubMed literature survey was done using “pregnant” “pregnancy” and “endocarditis” as the key words. All case reports published between 1981 and 2010 in English language were reviewed to study patient demography, causative organisms, predisposing factors, methods of treatment and clinical outcomes.

Results - A total of 41 case reports (43 pregnant women with IE) were reviewed. Mean age was 28 (±4.75) years. IE was diagnosed in their first, second and third trimesters in 5%, 31%, and 43% respectively while another 21% of the cases were diagnosed during postnatal period with 5 cases following abortions. The number of mothers diagnosed in 2nd and 3rd trimesters remains the highest and diagnosis of IE during postnatal period is also an important observation (Figure 1).

Conclusion - Pregnancy complicated by IE is associated with significant morbidity and mortality for both mother and foetus. Extreme care is necessary for pregnant women who were diagnosed with IE and should be managed in a tertiary centre with multidisciplinary specialist care.

Keywords: “Pregnancy” “endocarditis”

I. INTRODUCTION

Heart disease is one of the most important complications during pregnancy and is responsible for 10-15% of maternal mortality (Montoya, et al., 2003). Infective endocarditis (IE) during pregnancy is rare - the incidence has been estimated to be 0.006 % (ESC guidelines, 2009). However, this is a potentially lethal complication. The maternal mortality rate can reach to 33% - (Montoya, et al., 2003) with most deaths related to either heart failure or an embolic event. The rate of foetal mortality is also high – up to 29% (Montoya, et al., 2003).

Our aim was to study the risk factors and maternal and foetal outcome in cases of IE during pregnancy.

II. METHOD

A literature survey was done in PubMed using “pregnant” or “pregnancy” and “endocarditis” as key words. All the case reports published between 1981 and 2010 in English language were reviewed to find patient demography, causative organisms, predisposing factors, methods of treatment and clinical outcomes.

III. RESULTS

A total of 41 case reports with 43 cases of IE in pregnancy were reviewed. Mean age of the patients was 28 (±4.75) years. IE was diagnosed in 5% in their 1st trimester, 31% in the 2nd and 43% in the 3rd trimesters respectively while another 21% of the cases were diagnosed during postnatal period with 5 cases following abortions. The number of mothers diagnosed in 2nd and 3rd trimesters remains the highest and diagnosis of IE during postnatal period is also an important observation (Figure 1).
Figure 1: Diagnosed trimester

Prior history of cardiac pathology was known in 30% out of which rheumatic heart disease amounts for 62%, VSD 15% and PDA 8%. Among the other predisposing factors, history of IV drug abuse was present in 14% and 5% presented with history of previous prosthetic valve insertion. Nearly half of the cases had no reported history of pre-disposing factors (Table 1).

Table 1: Pre-disposing factors

<table>
<thead>
<tr>
<th>Pre-disposing factor</th>
<th>No. (%) of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 H/O IV drug abuse</td>
<td>6 (14)</td>
</tr>
<tr>
<td>2 Underlying cardiac pathology</td>
<td>13 (30)</td>
</tr>
<tr>
<td>3 Prosthetic valve</td>
<td>2 (5)</td>
</tr>
<tr>
<td>4 H/O dental surgery</td>
<td>1 (2.27)</td>
</tr>
<tr>
<td>5 None</td>
<td>22 (50)</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
</tr>
</tbody>
</table>

The commonest causative organisms according to species were *Streptococcus* and *Staphylococcus* species with 51% and 29% respectively. Infection with *Streptococcus viridans* was 31% and MRSA 9%. 11% were gram negative cases including infections with *Neisseria gonorrhoeae*, *Salmonella typhi*, *Haemophilus parainfluenzae* and *Enterobacter*. Three culture negative cases (8.6%) and two cases with multiple infections were also reported (Table 2).

Table 2: Causative organisms

<table>
<thead>
<tr>
<th>Organism</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta haemolytic streptococci</td>
<td>2.3</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>2.9</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>5.7</td>
</tr>
<tr>
<td>Gram positive cocci</td>
<td>5.7</td>
</tr>
<tr>
<td>Group B streptococcus</td>
<td>8.6</td>
</tr>
<tr>
<td>Haemophilus parainfluenza</td>
<td>2.9</td>
</tr>
<tr>
<td>Neisseria gonorrhoeae</td>
<td>2.9</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>2.9</td>
</tr>
<tr>
<td>Staphylococcus aureus (MRSA)</td>
<td>8.6</td>
</tr>
<tr>
<td>Staphylococcus aureus (MSSA)</td>
<td>20</td>
</tr>
<tr>
<td>Streptococcus viridans</td>
<td>31</td>
</tr>
<tr>
<td>Other streptococcus spp.</td>
<td>2.9</td>
</tr>
<tr>
<td>Culture negative</td>
<td>8.6</td>
</tr>
</tbody>
</table>

(MRSA: Methicillin resistant *Staphylococcus aureus*, MSSA: Methicillin sensitive *Staphylococcus aureus*)

Valve involvement was mitral valve 53%, aortic valve 33%, tricuspid 25% and pulmonary 2.8%. Involvement of two valves was present in 14% cases.

The mode of delivery was vaginal in 45% and caesarean section in 31% cases (not mentioned in 24%). Premature delivery rate was 14%.

Valve replacement surgery was done in 50% of the patients. 18% were done before delivery and 82% were done after. In all the cases with delivery by caesarean section, the valve replacement surgery was done soon after the delivery.

Foetal mortality was 7%. All were due to abortions/miscarriages and no cases of still births and post natal deaths were reported in this review.

The maternal mortality rate was 9.30%. Despite different methods of treatment, the rate of maternal complications also remained high in the reviewed IE cases.
There was a significant increase in the rates of Thromboembolism and septic embolism in last ten years. Cardiac complications were reduced in 2001-2010, when compared to 1981-1990 and 1991-2000. Nosocomial infections, Deep vein thrombosis, Multisystem failure were not reported in 1981-2000. But, 6.97%, 2.32%, 2.32% were reported in the year 2001-2010 respectively.

IV. DISCUSSION

IE is a rare condition to associate with pregnancy, However, it needs prompt diagnosis and early and effective treatment due to the risk of complications that affects maternal and foetal outcomes. Despite the number of different treatment modalities, IE in pregnancy results in high mortality and morbidity rates for both the mother and foetus.

The findings in this review show that the 3rd trimester is the most prone period to acquire this infection. Interestingly, there was a significant number of cases with IE during postnatal period, which also included a number of cases following abortions. Therefore special care should be taken to prevent IE following delivery as well.

The commonest causative organism is Streptococcus viridans. The Staphylococcus aureus infection and infections with Gram negative organisms were also high. Few cases were also reported to be culture negative. Therefore it was observed that both common and uncommon organisms could cause IE during pregnancy.

All the cases were managed promptly following diagnosis where half of the cases were managed conservatively without surgery. These patients were treated only by administration of intravenous antibiotics and the rest underwent valve replacement surgery in addition to the antibiotic therapy. The patients who underwent valve replacement surgery had good long-term prognosis despite the high risk of complications. Majority of the patients (nearly 80%) had valve replacement surgery after the delivery as many of the clinicians who treated these patients thought that this is the safest strategy for both the mother and the foetus.

The complication rates remained high in patients with IE during pregnancy. Thromboembolism, septic embolism and cardiac failure were the most common complications. Few cases were also
reported having post abortive endometritis, which shows a risk of developing IE as a result of concurrent gynaecological infection. Despite the high morbidity rates, the mortality rate of mothers was lower (9%) compared to the previously published data (ESC guidelines, 2009). Foetal mortality and morbidity were also lower, where all the foetal losses were due to abortions.

V. CONCLUSION

Pregnancy complicated by IE is associated with high morbidity and mortality for both mother and foetus. These patients should be treated with extreme care and prompt diagnosis and early and effective management is necessary by a multidisciplinary team in a tertiary care centre to minimise complications and improve maternal and foetal outcomes.

REFERENCES


HIV/AIDS Risk Reduction and Prevention among Drug Users through Behavioral Interventions in an Urban Setting

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Abstract— Drug use is a major risk factor in spreading HIV infection. Drug users (DUs) might trade sex for drugs or for money to buy drugs and/or vice versa. Drug use can reduce a person’s commitment to use condoms and practice safer sex. Often, substance users have multiple sexual partners. This increases their risk of becoming infected with HIV or another STI. Therefore, changing drug-related behaviors contributes to eradication of transmission of HIV. A behavior change (BC) model, which directly address BCs than merely conducting awareness/training workshops was implemented in Negombo, a tourist destination located in west coast of Sri Lanka where dwellers are vulnerable for drug use and sex trade. BC intervention tools used included low-cost community camps, group, ex-user and one-to-one discussions, brainstorming sessions to demystify myths about drug use and HIV while strengthening target groups. A rapid situation and response analysis was conducted prior to commencing interventions. Interventions concentrated more on 10 specific spots in Negombo. 350 DUs, their families, peers, 170 regular sex partners (RSP) including commercial sex workers (CSW), and neighbors were targeted through interventions. As a result, of BC model 80 quitted drug use, 59 reduced use, 29 changed their behaviors, 21 work as peer educators, 37 directed to STD clinics. 59 relapsed. The interventions with RSP resulted in following; 32 supported DUs to quit, 35 were educated on safer sex practices, 13 requested for condoms. BC model resulted in BCs in DUs, RSP, families and in their localities going beyond awareness and education. DUs and RSPs reduced individual risk behaviors, promoted and practiced safer sex practices (ex: condom use), motivated to get medical assistance for symptoms and suspected exposure to STDs and if engaged in risky behavior, to be tested. Changing behaviors related to drug use itself results in HIV risk reduction and prevention.

Keywords— HIV/AIDS, Behavior change, Drug Users

I. INTRODUCTION

Heroin is a semi synthetic opiate and it is a depressant (UNODC, 2013). According to the National Dangerous Drug Control Board, there are 45,000 heroin users in Sri Lanka and the estimated number of drug users has been stable over past years (NDDCB, 2013). The National STI/AIDS Control Programme reports that there were 1,845 cases of HIV and 491 AIDS cases reported by last quarter of 2013 and during the year 2013, 196 new cases of HIV were reported.

Drug use is a major risk factor in spreading HIV infection. Injecting drug use is also risk factor in spreading HIV due to sharing of needles. However, in Sri Lanka the risk of injecting drugs and sharing needles is very insignificant given very low level injecting drug users in the country. The heroin intake is mostly through inhalation also known as “chasing the dragon” among the drug users and only 2% of the heroin users are injecting drug users (Senanayake and Lakmini, 2012). Only one case has been reported as an HIV transmission due to injecting drug use thus far (Senanayake and Lakmini, 2012).

Drug users (DUs) might trade sex for drugs or for money to buy drugs and/or vice versa. Drug use can reduce a person’s commitment to use condoms and practice safer sex. Often, substance users have multiple sexual partners. This increases their risk of becoming infected with HIV or another STI.

In order to reduce the risk of HIV/AIDS among drug users and their regular sex partners, it is important to address the issue of drug use, as mentioned above, drug use play big role in unprotected sex. While directing the interventions to stop or free the heroin users from the drug users, awareness on
HIV/AIDS and other STIs and also regularly testing for any kind of STI are of utmost importance.

A behavior change model, which directly address behavior changes than merely conducting awareness/training workshops was implemented in Negombo by Alcohol and Drug Information Centre (ADIC). Negombo is a tourist destination bordered by a lagoon located in west coast of Sri Lanka. Negombo is in the second largest population centre in Sri Lanka which is the Gampaha district. 11% of the population in Gampaha district is concentrated in Negombo having a population of 121,933 (Negombo, 2013). The main occupations of residents of Negombo are fishing and sale of fish. In addition, tourism also provides a livelihood to many as Negombo is a major tourist attraction. One main cause of poverty among this population is heroin use and many social issues arise as the users are involved in illegal activities like stealing, robberies and sex trade. It is identified that there is a high risk of HIV/AIDS and sexually transmitted infections (STIs) too.

II. CONCEPT

There are many myths and beliefs attached to heroin use. The interventions carried out in Negombo were focused on revealing the reality of the heroin use and critically analyzing the factors that promote drug use while addressing the factors that compel users to continue the usage.

Heroin is a depressant (UNODC, 2013). A depressant cannot stimulate the body and generate happy feelings rather it depress the body based on its chemical properties. Therefore there is a discrepancy in claimed, reported or expected experience and the actual behavior of the chemical. Today science has proved that heroin results in the same manner as any depressant.

Freeing involves not just removing a person from the drug but also teaching about external factors which facilitate continuation of drug use and developing the person to face the forces that compel him to restart.

III. METHODOLOGY

A rapid situation and response analysis was carried out in Negombo to identify the locations where the intensive interventions needed to target the risk population. As per the analysis, Pitipana, Wella, Mankuliya, Daluwakotuwa, Cannel Road, Dalupotha, Dehimalwatta, Harischandrapura, Jeen Junction and Kadirana were identified as hotspots.

The interventions carried out are based on a socio-psychological approach, which includes the individual and the environment. Its ultimate objective is to free the heroin user from drug use. One key factor in this approach is that it does not make the user paranoid or scared about the drug or the process of being free from the drugs. And also it does not attempt to move the drug user from heroin to another drug. Rather a dialogue is initiated with the user without removing him or her from their environment/setting with the objective of critically analyzing the drug use, the initiation factors, expectations and the actual experience with the user. The process empowers the user to identify the internal and external factors that facilitate the continuation of drug use while making the freeing or quitting difficult for the user.

350 drug users, their families, peers, 170 regular sex partners (RSP) including commercial sex workers (CSW), and neighbors were targeted through interventions. Following activities were carried out as interventions in the community.

A. 10-15 Day Low Cost Camp

This camp is organized for the drug users who are willing to be free from heroin use. It is important to have camp members from the same locality. All the camp members discuss about the factors that compel them to continue the drug use and plan their response towards those once they are back in the community.

B. One to One Discussions With Heroin Users

Field Workers meet heroin users individually in their locations. In these one to one meetings barriers to quit (physical, psychological and social), myths on
heroin use and how to overcome the withdrawal symptoms are mainly discussed. Especially private and sensitive matters related to heroin use and their personal lives are openly revealed by the heroin users in these meetings.

C. Make the Support Environment to be Free

Family members of heroin users (parents, wife, and children etc.), regular and commercial sex partners, community members and leaders of their own community, police, and religious leaders are the main factors of the social aspect. They play a very important supportive role of this treatment concept. Trainings and discussions with them are used to create the friendly and supportive environment for heroin users to quit.

D. Support Groups

Support groups are formed by ex-drug users. Ex-drug users are defined as heroin users who have stopped the heroin intake for minimum of six months. The main responsibility of the support group is to help current drug users in their own community to quit the drug use. The experience of ex-drug users is important in the process of making the current drug users stop the drug use. The main benefit for these support group members—ex users is that the approach strengthens them to continue quitting by helping others.

E. Small Group Discussions with Ex-Drug Users

Ex-drug users were facilitated by ADIC team to talk openly regarding the positive benefits they received after stopping heroin use in the meetings. This process helps everyone to learn positive practices of others which will sustain and boost their efforts of stopping.

F. Group Meetings Mixed with Ex Drug Users and Current Users

Mixing both groups motivate them to share their experiences. Questions about barriers to quit heroin use are answered by the ex-drug users. The current users discuss the questions, problems and doubts they have with the ex-users and the process help them to strengthen their ideas to quit.

G. Brain-Storming Discussions in User Groups

User group discussions were focused mainly on how and what users feel after using the drug and the withdrawal symptoms. In many cases, the reported withdrawal symptoms were different and contradictory although the users have used the same drug at same level in the same social background. Using such examples, users were able to differentiate learned effects of the drug from the subculture and the real chemical effects of the drug.

H. Group Discussions with Vulnerable Groups

Impish youth who are very closely engaging with user groups or living in same drug using community and who are not using heroin are recognized as vulnerable groups. They are at a severe risk to initiate heroin use with the force of their user friends. Changing their thinking pattern regarding heroin use and prevent them of starting heroin use are the main purposes of working with these groups.

I. Increasing the Range of the Happiness and Push Drug Users and Ex-Drug Users towards a Healthy Life

Sports, musical events and other recreational events are organized to make the users realize about many avenue for happiness. And also as an initiative to develop their personalities, responsibility of organizing these events is given to them.

J. Follow Ups

During the follow ups carried out by ADIC team, concerns about stopping use, reducing use, behavioral changes, environmental changes, withdrawal symptoms are discussed and solutions are derived.

IV. RESULTS

Behavior change interventions implemented resulted in BCs in DUs, RSP, families and in their localities going beyond awareness and education. DUs and RSPs reduced individual risk behaviors, promoted and practiced safer sex practices (ex: condom use), motivated to get medical assistance for symptoms and suspected exposure to STDs and if engaged in risky behavior, to be tested. Changing behaviors related to drug use itself results in HIV risk reduction and prevention.

As a result, of behavioral change model employed in Negombo, 80 drug users quitted drug use, 59 reduced the usage, 29 changed their behaviors, 21started working with ADIC team as peer educators and 37 were directed to STD clinics. 59 drug users relapsed. The interventions with RSP resulted in following; 32 supported drug users to
quit, 35 were educated on safer sex practices, 13 requested for condoms. Discussions with the field staff provide evidence that quitting or being free from heroin use is not a difficult task as portrayed by the society and the drug trade.

V. CONCLUSION

The effectiveness of this approach lies on the fact that it considers all the facets of addiction including chemical and psychological effects. In most instances, the users are attached to the psychological factors like the feeling of belonging to a group rather than the real chemical effect. Therefore changing the behavior of drug use requires changing the internal and external factors that compel the user to continue. Once these factors are addressed, an environment which does not demand drug use is created. This environment supports the users who are not yet empowered to tackle internal factors that promote heroin use to them. Similarly when internal factors are properly addressed, immunity is created within the user not initiate drug use again even in an environment where factors that trigger continuation exist.

Another interesting fact of this approach is that when an immunized and empowered individual is made; it creates a trend of being free similar to the trend which promoted the drug use. The methodology implemented in Negombo requires less effort and budget as the heroin users are kept in their own locality rather than in a rehabilitation centre environment. Another advantage is that users are empowered and immunized to face the factors that compel him/her to restart the usage once they are back in the community.

This behavior change model can be adopted in any setting where there exist a committed individual or an organization to conduct interventions for a period of one year and then carry out follow ups.

ACKNOWLEDGMENT

The Alcohol and Drug Information Centre (ADIC), Mr. Pubudu Sumanasekara the Executive Director, staff and volunteers need to be acknowledge for their support in carrying out this documentation of an intervention carried out by ADIC.

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2 Mahesh Nissanka is a Project Coordinator attached to Alcohol and Drug Information Centre (ADIC). He is responsible for coordinating, monitoring and supervising the Help to be Free from Heroin Use programme at ADIC. In addition he is the Project Coordinator of the Drug User component of Global Fund HIV/AIDS Risk Reduction Round 9 Project from 2012 to present.
Can Clomiphene Citrate Improve the Quality of Seminal Fluid Parameters in Idipathic Oligoasthenoteratozoospermic Males

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Abstract

Introduction: Male infertility is a rising problem worldwide. Idiopathic male infertility is responsible for 31% of all male infertility. Among them 44% is due to Idiopathic Oligo Asthen Teratozoospermia (iOAT) syndrome. The role of medical treatment in managing iOAT syndrome is controversial. But there are accumulating evidence of the efficacy of Clomiphene citrate on treating iOAT syndrome.

Objective: To determine the efficacy of Clomiphene Citrate therapy in improving seminal fluid parameters of a group of male patients with iOAT syndrome.

Study type: a comparison study was done using retrospective data.

Method: This study was conducted in the Reproductive biology laboratory of the Faculty of Medicine, Galle. Medical records of 52 patients presented with iOAT syndrome, treated with clomiphene citrate were screened using the exclusion and inclusion criteria. The pre-treatment and post-treatment seminal fluid analysis of the patients were compared using the paired t-test.

All the patients were given clomiphene citrate 50mg per day in divided doses for minimum of 3 months. Post-treatment seminal fluid analysis were done at the end of the 4th month of commencing the treatments.

Results: Sperm concentration, sperm total count, normal morphology, motility and viability are improved significantly (p<0.001). The improvement of the volume is not significant (p> 0.001)

Conclusion: Clomiphene citrate improves the sperm concentration, total count, normal morphology, motility and viability in a group of selected male patients with iOAT syndrome but the volume.

Keywords— Clomiphene citrate, iOAT, Male infertility

I. INTRODUCTION

Infertility, a rising global problem affected 48.5 million couples in reproductive age worldwide in 2010 (Mascarenhas et al. 2012) (Winters and Walsh 2014). Though the recent demographic data on this issue is not available, the prevalence of subfertility among Sri Lankan couples in reproductive age is about 15%(Winters and Walsh 2014) (Farouk Mahmoud 2011).

In General the male factors contribute to about 50% of all infertility cases(A. Jungwirth et al. 2013) (Irvine 1998). The common causes of male infertility are Idiopathic oligoasthenoteratozoospermia (iOAT), Varicocele, Hypogonadism and genetic disorders, mal-descended testes , Autoimmune antibodies and urogenital infections (A. Jungwirth et al. 2013).

However iOAT is considered as the major cause of male infertility worldwide and its prevalence is about 30% of all male infertility (A. Jungwirth et al. 2013) (Gudeloglu, Brahmbhatt, and Parekattil 2014).

Though the medical or surgical management for male infertility is very time consuming, the success rates are fairly good if the causes for the infertility is known (Gudeloglu, Brahmbhatt, and Parekattil 2014). But the majority of male infertility is either due to idiopathic causes(30%) or due to multiple causes (A. Jungwirth et al. 2013) . The outcome of the medical management for these cases is not promising (Gudeloglu, Brahmbhatt, and Parekattil 2014) . Therefore the outcome of medical management for male factor infertility, as a whole has a fairly low success rate which discourages not only patients but also physicians. Further more, in most of the instances, the availability of modern
Advanced Reproductive Technologies (ART) which have more reliable and better success rates has become the first line of the management of male infertility which is not rational when social and economic impacts are considered.

Though several drugs are available in the market for male factor infertility, Food and Drug Authority (FDA) has approved only Gonadotrophin Releasing Hormone (GnRH), Human chorionic-gonadotropin (hCG), Human menopausal Gonadotropin (hMG), Highly purified or recombinant human Follicle-Stimulating Hormone (rhFSH) and Dopamine agonist (Gudeloglu, Brahmbhatt, and Parekattil 2014) (A. Jungwirth et al. 2013). The other drugs such as Aromatase inhibitors, anti oxidants, Co-Enzym Q, Selective estrogen receptor modulators (Clomiphene Citrate) have been categorized as remedies or empirical and are being used as off label drugs (A. Jungwirth et al. 2013).

However some recently published literature has shown that clomiphene citrate (beta-diethyl aminoethoxy) can improve seminal fluid parameters and pregnancy rates in males with iOAT. Though there are several protocols of administration of clomiphene citrate, the widely used protocol is 25mg per day as a single dose (Patankar et al. 2007) (Hayashi et al. 1988). Though it has not been clearly documented, the duration of clomiphene citrate treatment is thought to be more than three (03) months to have a detectable response (Patankar et al. 2007) (Hayashi et al. 1988).

Clomiphene is an estrogen receptor inhibitor at the level of hypothalamus, inhibiting the negative feedback of estrogen on GnRH release. Clomiphene can up-regulate the hypothalamic–pituitary–gonadal axis in males increasing the serum Follicular stimulating Hormone (FSH) and testosterone levels (“DrugBank: Clomifene (DB00882)” 2014).

In 1988, Hayashi N et.al, noticed statistically significant improvement of sperm motility and sperm count in a group of Japanese males with idiopathic infertility with clomiphene citrate treatment. But they haven’t recorded an improvement of sperm morphology. However four (04, 10%) spontaneous pregnancies have been recoded in their study sample (n=40) (Hayashi et al. 1988).

According to findings of Patankar SS et al., in 2007, clomiphene citrate has ability to improve sperm count, sperm motility but sperm morphology to certain extent in a group of oligozoospermic patients (Patankar et al. 2007).

Based on the review study done by Willets AE, in 2012, “there is insufficient evidence to indicate that clomiphene is effective for the treatment of male infertility”. However the author agreed that the majority of the studies have demonstrated a statistically significant increase in sperm concentrations (Willets AE, Corbo JM, and Brown JN 2013).

In 2012, Iqubal Mirza has recorded an improvement of semen volume, sperm count, sperm motility and to a certain extent sperm morphology in his study group after treating with clomiphene citrate. It was a quasi-experimental study and the study population was 50 males with iOAT (Zahoor Iqbal Mirza).

The objectives of the present study were to study the effect of Clomiphene Citrate on patients with iOAT syndrome. This study was carried out in the Reproductive Biology Laboratory (RBL) of the Faculty Of Medicine, University of Ruhuna. RBL of the Ruhuna Medical Faculty is a center of excellence in the management of male factor infertility. It has the country’s first sperm bank and it caters to patients who seek help from all over the country.

II. PATIENTS AND METHODS

This was a retrospective study conducted in the Reproductive Biology Laboratory (RBL), Faculty of Medicine, Karapitiya. Medical records of the males treated with clomiphene citrate, 25 mg daily for more than 3 months, between 2009 and 2012 were selected from the data base. The medical records were carefully evaluated and screened by an Andrologist and the records that are complying with the following inclusion and exclusion criteria were included for the analysis.

**Inclusion Criteria:**

1. Males, who's Basic Seminal fluid Analysis (SFA) assessments were done in the RBL.
2. Males who had pre and post treatment SFA assessments.
3. Infertile men having OTS (Sperm concentration < 15\times 10^6 per ml., Morphology <30% using standard protocol, Motility (a+b) < 50%)
4. Otherwise healthy males aged 25-45 having coital frequency more than 4 times/week with the married partner.
5. Normal levels of serum FSH, LH, and testosterone.
6. Married and having a stable relationship for more than 3 years.

Exclusion criteria:
1. Males who were on long term (more than 6 months) medical treatment.
2. Males with past history of any surgical intervention related to scrotum or testes.
3. Previous history of mumps, orchitis, trauma to testes, cryptorchidism or varicocele.
4. Previous history of STD.
5. Previously treated for infertility.

The data was analyzed by Epi Info 7. Two tailed P test was used to compare pre – treatment and post – treatment groups.

III. RESULTS AND DISCUSSION

Fifty one (51) patients were in the study. The mean age of the study population was 33 (SD ± 3.5). The mean FSH, LH and testosterone values of the study sample prior to treatment were 2.7 (SD ± 1.32), 3.5 (SD ± 1.6) and 4.6 (SD ± 2.3) respectively. The mean testicular volume was 28 ml.

The mean semen volume of the pre-treatment phase was 2.15 ±1.3 ml. After treating with clomiphene it was 2.42 ± 0.89 ml. It doesn’t show any significant improvement.

The mean concentration of the pre-treatment group was 6.17 \times 10^6 (SD ± 5.59)per/ ml. It showed a statistically significant improvement (p<0.001) after clomiphene treatment. The mean concentration of the post – treatment group was 18.18 \times 10^6 (SD ± 11.1).

Morphology also showed a significant improvement (p < 0.001) with clomiphene treatment. Prior to the treatment the mean morphology was 22.4 ± 8.8 and it was 29.5 ± 10.7 after the treatment.

The mean motility of the pre-treatment group was recorded as 32.5 ± 14.9. In post-treatment group the mean motility was 45.3 ± 14.7. It showed a statistically significant improvement (p < 0.001).

In the post-treatment group, the mean viability was 56.9 ± 12.6. It too showed a statistically significant improvement (P < 0.001) when compared to the mean viability of the pre-treatment group, 44.6 ± 14.

Table 1: Summary of the statistical analysis of the various Seminal Fluid (SF) parameters in pre and post study groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre Treatment</th>
<th>Post Treatment</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Volume</td>
<td>2.15</td>
<td>2.42</td>
<td>0.89</td>
</tr>
<tr>
<td>Concentration</td>
<td>6.17</td>
<td>18.18</td>
<td>11.1</td>
</tr>
<tr>
<td>Total Count</td>
<td>13</td>
<td>48.6</td>
<td>18.5</td>
</tr>
<tr>
<td>Morphology</td>
<td>22.4</td>
<td>29.5</td>
<td>10.7</td>
</tr>
<tr>
<td>Motility</td>
<td>32.5</td>
<td>45.3</td>
<td>14.7</td>
</tr>
<tr>
<td>Viability</td>
<td>44.6</td>
<td>56.9</td>
<td>12.6</td>
</tr>
</tbody>
</table>

The results showed significant improvements of concentration (pre treatment 6.17 ± 4.59 and post treatment 18.18 ± 11.1), total count (pre treatment 13 ± 12.2 and post treatment 48.6 ± 18.5), morphology (pre treatment 22.4 ± 8.8 and post treatment 29.5 ± 10.7), motility (pre treatment 32.5 ± 14.9 and post treatment 45.3 ± 14.7) and viability (pre treatment 44.6 ± 14 and post treatment 56.9 ± 12.6). But volume has not showed a significant improvement (pre treatment 2.15 ± 1.13 and post treatment 2.42 ± 0.89).

Our results are compatible with the results of Mahmoudreza Moradi et. al. 2010 (Mahmoudreza Moradi et al. 2010). They have reported statistically significant improvements in concentration, total count, morphology, motility and viability but the volume.

In 2012 Zahoor Iqbal Mirza et. al. Recorded a significant improvement of the the SF volume after clomiphene treatment (Zahoor Iqbal Mirza) which we could not observe.
IV. CONCLUSION AND RECOMMENDATIONS

Clomiphene citrate can improve the sperm concentration, total count, normal morphology, motility and viability in a group of selected male patients with iOAT syndrome. A well designed randomized clinical trial is recommended to study the effects of clomiphene citrate on iOAT.

REFERENCES


Hypocholesterolaemic Effect of Okra on Cholesterol Induced Wistar Rats

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Abstract
Okra or Lady's fingers (Hibiscus esculentus) which belongs to the Family Malvaceae is a mucilaginous vegetable frequently included in the diet of Sri Lankans. There is currently great deal of interest in the hypoglycaemic effect of various vegetables. It has been reported that the cholesterol lowering effect of H. esculentus in Senegalese adult men. Abelmoschus esculentus (L.) Moench., synonym of H. esculentus which is available in all over the world, popular and has been claimed to have various health benefits and include anti-diabetic properties. According to our studies it shows that okra fruit possesses hypoglycaemic activity and the anti-hyperglycaemic compound present in okra fruit is heat labile. The aim of this study was to investigate the hypocholesterolaemic effects of the water extract of okra fruit on Wistar rats. Weanling male Wistar rats (100g – 150g) were separated in to groups (test and control) with 8 rats in each group. Hypercholesterolaemia was induced by giving a diet containing 10% butter (Highland), 90% WHO recommended rat and mouse feed pellets and water ad libitum for 28 days. Induction of Hypercholesterolaemia was verified after 28 days by measuring fasting (12-14hrs) blood cholesterol levels. These animals were used as positive control and for the test groups. The negative control animals were fed on WHO recommended rat and mice feed formula and water ad libitum. The positive control group and the test groups were given hypercholesterolic diet continuously while the test groups were orally feeding the water extract of okra (with 500 and 1000mg/Kg dose) for 7 days. After one week the rats were fasted (12-14hrs) and anesthetized and blood was collected from tail vein for determination of fasting serum cholesterol levels. All the results were compared by using the Student’s t-Test in Microsoft Excel. Administration of water extract of Okra at the dose of 1000mg/Kg showed significant reduction (p<0.05) in the level of serum cholesterol in Wistar rats while the dose 500mg/kg was not. Therefore okra can be used as a vegetable with hypoglycaemic and hypocholesterolic properties.

Keywords - Okra, Hibiscus esculentus, Abelmoschus esculentus

I. INTRODUCTION
Okra Abelmoschus esculentus (L.) Moench., synonym of Hibiscus esculentus or Lady's finger which belongs to the Family Malvaceae is a flowering plant and is cultivated throughout the tropical and temperate regions of the world. Sri Lankan name of Okra is Banndakka and other names of Okra are Okro, Ochro, Okoro, Quimgombo (Cuba), Quingumbo, Gombo, Kopi Arab, Kacang Bendi, Bhindi (South Asia), Bendi (Malaysia), Bamia, Bamyia or Bamiieh (Middle East), Gumbo (Southern USA), Quiabo, Quiabos (Portugal and Angola), okura (Japan) and Qiu Kui (Taiwan). Okra traces its origin from what was known as Abyssinia (Ethiopia) spreading right through to Eastern Mediterranean, India, Africa, North America, South America and the Caribbean. Though long popular in the South, it is becoming increasingly common and well known in Western Countries (http://newedgepublishing.com).

In 1977 it has been reported the effect of okra (Hibiscus esculentus) mucilage on the plasma cholesterol level in rats. There is no clear literature based on this study (Woolfe, J. A. (1997). It has been reported that the cholesterol lowering effect of okra (Hibiscus esculentus) in Senegalese adult men. It is a fruit high in water-soluble fibre and widely consumed in Africa investigated as a potential candidate to decrease cholesterol (Bangana et al., 2005). The extracts from total plant
of by dichloromethane or methanol and extracts from fruit by dichloromethane or methanol possessed hypolipidemic activity in tyloxapol-induced hyperlipidaemia in mice. (Huynh Ngoc, H. et al. 2008). It has been reported the antidiabetic and antihyperlipidemic potential of Abelmoschus esculentus peel and seed powder (AEPP and AESP) in streptozotocin (STZ)-induced diabetic rats. Administration of AEPP and AESP at 100 and 200 mg/kg dose in diabetic rats showed significant reduction in blood glucose level and increase in body weight than diabetic control rats. This study results the antidiabetic and antihyperlipidemic potential of A. esculentus peel and seed powder in diabetic rats (Sabitha, V. et al., 2011).

This study was undertaken to investigate the effects of water extract of okra on plasma cholesterol level on Wistar rats. The water soluble fraction was studied first as the related studies are mostly based on it.

II. MATERIALS AND METHODS

A. Collection of plant materials
The fruits of H. esculentus were obtained from the local market of Kelaniya, Sri Lanka. A specimen of the plant and fruit was deposited in the National Herbarium, Department of National Botanic Gardens, Peradeniya, Sri Lanka after identification of the plant by a botanist.

B. Animal model
The feeding trials were conducted using out bread Wistar rats (originally from the Clea animal breeding company, Tokyo, Japan). The colonies have been bred and maintained at the Animal Center of Medical Research Institute, Colombo, Sri Lanka for 10 years. Weanling male Wistar rats (4 weeks) were separated in to groups (test and control) with 6 rats in each group. The animals (150g – 200g) were housed separately and the groups are selected so that the average weights in each group were similar. The rats were fed on WHO recommended breeding feed formula (Sabourdy, 1988). The test group was given the water extract of the fruit of okra for one week. The rats were fed with the standard WHO feed, water ad libitum maintained under standard conditions at Animal Center, University of Sri Jayawardenepura. The oral administration was done by using Sondi needles.

C. Preparation of the water extract of the fruit
Fresh fruits of okra were collected from a local market of Kelaniya, Sri Lanka. Then, the pods were thoroughly washed with distilled water, cut into small slices by a sharp knife. About 1Kg of the sliced pods was crushed by a blender. The mixture was then stirred gently for 10 to 15 minutes with a glass rod; filtered using a thin layer of cotton to remove the insoluble matters and filtrate was collected. Water extract was freeze-dried using the freeze-dryer to obtain dry sample.

D. Animal doses
Animal doses were derived by considering the dry extract powder in mg to the body weight (Kg) of the rats. Freeze-dried water extract powder was dissolved distilled water to make the water extractives. Okra extractives were given in two different doses separately for each group. The two doses, 500, 1000, (mg/Kg body weight) was orally administered to the test group of rats (n=8) for one week. The control group was given water.

E. Collection of fasting blood cholesterol and separation of serum
Animals were fasted for 12-14 hours and anaesthetized using diethyl ether. The blood samples (0.5 ml) were collected by tail vein puncture. Clear, non–haemolyzed serum was separated by centrifugation at 3000rpm for 10 minutes using a centrifuge. (Jawaki CFM-100, Japan). The cholesterol concentrations were analyzed immediately.

F. Determination of fasting blood cholesterol
The serum samples were analyzed by kits commercially available cholestrol esterase and cholesterol Oxidase Reagent (Pointe cholesterol (liquid) reagent, Canton, USA).Cholesterol (liquid) reagent (1ml) was pipette into labeled tubes followed by the addition of sample, control or standard (0.01ml each). The tubes were incubated at 37°C and absorbance was measured in the spectrophotometer (Shimadzu-UV200, Japan) at 500nm.

G. Hypercholesterlomic inducer
Butter was used as the hypercholesterlomic inducer. The animal feed was used for induction of hypercholesterlomia in rats. Hypercholesterolaelma was induced by giving a diet containing 10% butter (Highland), 90% WHO recommended rat and mice feed pellets and water ad libitum for 28 days. Induction of hyperlipidemia was verified after 28 days by measuring fasting (12-14hrs) blood cholesterol levels.
II. EXPERIMENTAL DESIGN

Weanling male Wistar rats (100g – 150g) were separated into groups (test and control) with 8 rats in each group. The negative control animals were fed on WHO recommended rat and mice feed formula and water ad libitum (Sabourdy, 1988). Hypercholesterolaemia was induced by giving a diet containing 10% butter (Highland), 90% WHO recommended rat and mice feed pellets and water ad libitum for 28 days. Induction of Hypercholesterolaemia was verified after 28 days by measuring fasting (12-14hrs) blood cholesterol levels. These animals were used as positive control and for the test groups. The positive control group and the test groups were given hypercholesterolic diet continually.

The test group was given water extracts of okra (with 500 and 1000mg/Kg dose) and water ad libitum for 7 days. The Freeze-dried sample was dissolved in water to obtain the extraction. Animal doses were derived by considering the dry extract powder in mg to the body weight (Kg) of the rats. Extractives were freshly prepared daily and the aliquots were taken each day to administer the rats orally by using sondi needles. Okra doses of 500, 1000 (mg/Kg body weight) were administered separately to the test groups of rats (n=8) for one week. The control group was given water. At the end of the time duration (1week) the blood samples were collected.

III. STATISTICAL ANALYSIS

All the results are presented as Mean ±S.E.M. Data pairs, are compared by using the Student’s t-Test in Microsoft Excel. Difference will be considered if p<0.05

VI. RESULTS

<table>
<thead>
<tr>
<th>Doses (mg/Kg body weight)</th>
<th>Blood cholesterol levels (mg dl⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative Control</td>
</tr>
<tr>
<td>500</td>
<td>54.2±8.4</td>
</tr>
<tr>
<td>1000</td>
<td>44.4±7.7</td>
</tr>
</tbody>
</table>

n=8, p*<0.05, ±=standard error of mean

P values for blood cholesterol levels were significantly different for the dose of 1000 (mg/Kg body weight). Administration of water extract of Okra at the dose of 1000mg/Kg showed significant reduction (p<0.05) in the level of serum cholesterol in Wistar rats.

V. DISCUSSION

Hypercholesterolaemia leads many health problems in worldwide which cause important risk factors like atherosclerosis, stroke etc. Many hypercholesterolic drugs have already been proved to be useful in lowering serum lipid levels in patients. However, its side effects in long-term treatment have been frequently reported and its prices are still expensive. Thus, efforts to develop effective and better hypocholesterolic drugs had led to the discovery of natural agents (Huynh Ngoc, T., 2008). The hypocholesterolic activity of water extract of okra fruit (Abelmoschus esculentus) was studied on high fat diet induced models of Hypercholesterolaemia in Wistar rats. Hypercholesterolaemia was induced by giving a diet containing 10% butter (Highland), 90% WHO recommended rat and mice feed pellets and water ad libitum for 4 weeks. Induction of Hypercholesterolaemia was verified after 4 weeks by measuring fasting blood cholesterol level. Water extract showed significant hypocholesterolic effect by lowering the serum cholesterol levels. Administration of water extract of Okra at the dose of 1000mg/Kg showed significant reduction (p<0.05) in the level of serum cholesterol in Wistar rats while the dose 500mg/kg was not. Therefore water extract of okra can be used as a drink as well as vegetable with hypoglycaemic and hypocholesterolic properties.

VI. CONCLUSION

In conclusion, water extract from the fruit of Abelmoschus esculentus possessed hypocholesterolic activity in butter-induced Hypcholesterolaemic in rats. Water extract showed significant hypocholesterolic effect by lowering the serum cholesterol levels.
ACKNOWLEDGEMENT
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BIOGRAPHY OF AUTHORS

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4 Author is the Director of ITI, Colombo, Sri Lanka. His research interest is on Natural Products Chemistry & Chemical Biology biochemistry. He has produced more than 150 referred international and local journals. Dr. G.A.S. Premakumara has supervised 7 MsC and 6 PhD projects and many undergraduate projects.
In Vitro Lipase, Cholesterol Esterase and Cholesterol Micellization Inhibitory Activities of Ceylon Cinnamon (Cinnamomum Zeylanicum Blume)

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Abstract—Hyperlipidaemia is a group of metabolic disorders characterized by the elevated levels of serum triglycerides and cholesterol. It contributes significantly in pathogenesis of diabetes, obesity, hypertension and cardiovascular diseases. Hence, there is an imperative need for development of antilipidemic agents preferably from natural sources. Cinnamomum zeylanicum Blume is indigenous to Sri Lanka and used as a spice in several countries. According to some Sri Lankan traditional physicians the bark of this plant is claimed to possess antilipidemic effects by inhibition of lipid digestion and/or absorption. This study was initiated to investigate antilipidemic potential of Ceylon cinnamon in vitro.

Ethanolic (95%) and 1:1 dichloromethane: methanol (DCM:M) bark extracts of Ceylon cinnamon were used in this study. Different concentrations of ethanolic and DCM:M bark extracts (anti-lipase: 3.75 - 600 µg/ml, n = 3; cholesterol esterase: 3.125 - 100 µg/ml, n = 3; cholesterol micellization: 0.25 - 1 mg/ml, n = 6) were used in testing of antilipidemic effects.

The results revealed that both extracts possess moderate lipase inhibitory activity (ethanol bark IC₅₀ 301.09 ± 5.73 µg/ml and DCM:M bark IC₅₀ 297.57 ± 11.78 µg/ml), high cholesterol esterase inhibitory activity (ethanol bark IC₅₀ 30.62 ± 1.67 µg/ml and DCM:M bark IC₅₀ 34.39 ± 0.91 µg/ml) and moderate to high cholesterol micellization inhibitory activities (mean percentage inhibition of cholesterol solubility in micelles in ethanolic bark and DCM:M bark 98.09 ± 1.25 and 73.94 ± 1.95, at 1 mg/ml; 69.48 ± 1.99 and 62.15 ± 2.37 at 0.5 mg/ml and 49.48 ± 1.90 and 19.36 ± 4.57 at 0.25 mg/ml respectively). Further, anti-lipase and anti-cholesterol esterase activities were dose dependent.

It is concluded that Ceylon cinnamon bark possess anti-lipase, cholesterol esterase and cholesterol micellization inhibitory activities. This is a novel finding having therapeutic potentials and indicates the potential of using bark as a functional food for hyperlipidaemia.

Keywords—Ceylon cinnamon, bark extracts, lipid lowering, Cinnamomum zeylanicum

I. INTRODUCTION

Hyperlipidaemia is a group of metabolic disorders characterized by the elevated levels of serum triglycerides and cholesterol (Jacobson et al., 2007). It contributes significantly in pathogenesis of diabetes, obesity, hypertension and cardiovascular diseases (Fauci et al., 2008). Several factors, such as diet high in saturated fats and cholesterol, lack of physical activity, stress and life style factors play a significant role in development of hyperlipidaemia and related chronic diseases.

Currently, available antilipidaemic drugs to treat hyperlipidaemia include HMG-CoA reductase inhibitors, cholesterol absorption inhibitors, bile acid sequestrants and omega -3 fatty acids (Fauci et al., 2008). These drugs are very effective in management of hyperlipidaemia and related chronic diseases. However, most of these drugs are expensive and beyond the reach of many persons in developing countries and some of these drugs are associated with undesirable side effects such as myalgias, arthralgias, elevated liver enzymes, elevated blood glucose, dyspepsia and constipation (Fauci et al., 2008). Research conducted in recent years has shown that many of the natural products having antilipidaemic activity via multiple mechanisms (Adisakwattana et al., 2012; Kumar et al., 2011; Uahiyma et al., 2011; Ikeda et al., 2010).
These natural products are proven to be safe and less expensive compared to the available antilipidaemic drugs.

Cinnamomum zeylanicum Blume is indigenous to Sri Lanka and used as a spice in several countries. According to some Sri Lankan traditional physicians the bark of this plant is claimed to possess antilipidemic effects by inhibition of lipid digestion and/or absorption. Scientifically antilipidaemic properties of cinnamon have shown in various in vitro (Sheng et al., 2008) and in vivo models (Ranasinghe et al., 2012; Lee et al., 2003; Sambaiah & Srinivasan, 1991). However, antilipidaemic properties of Ceylon cinnamon via anti-lipase activity, cholesterol esterase and cholesterol micellization inhibitory activities were not previously reported. In this connection this study was initiated to investigate antilipidemic potential of Ceylon cinnamon via in vitro anti-lipase activity, cholesterol esterase and cholesterol micellization inhibitory activities.

II. MATERIALS AND METHODS

A. Materials

Alba grade cinnamon bark samples were collected from L.B spices (Pvt) Ltd, Aluthwala, Galle, Sri Lanka and G. P. De Silva and Sons spices (Pvt) Ltd, Ambalangoda, Sri Lanka.

B. Chemicals and equipments

Porcine pancreatic lipase (PPL, type II), p-nitro phenyl butyrate (p-NPB), pancreatic cholesterol esterase, oleic acid, phosphatidylcholine, cholesterol, epigallocatechin gallate (EGCG) and taurocholic acid were purchased from Sigma-Aldrich, USA. Cholesterol test kits were purchased from Fortress diagnostics, UK. All the other chemicals and reagents were of analytical grade.

C. Preparation of bark extracts of Ceylon cinnamon

Preparation of ethanolic bark extracts: Powdered bark (20 g) was extracted into 200 ml of 95% ethanol for 4-5 h and 4-6 cycles in a soxhlet extractor till the solvent in the Siphon tube and extractor becomes colourless. The extract was then filtered and evaporated under reduced pressure and freeze dried. The freeze dried extracts were stored at -20°C and used for the following assays.

Preparation of dichloromethane:methanol (DCM:M) bark extracts: Powdered bark (20 g) was extracted into 200 ml of dichloromethane:methanol in a ratio of (1:1 v/v) at room temperature for 7 days with occasional shaking. The extract was filtered and evaporated under reduced pressure and freeze dried. The freeze dried extracts were stored at -20°C and used for the following assays.

D. Anti-lipase activity of Ceylon cinnamon

Pancreatic lipase inhibitory activity of bark extract of Ceylon cinnamon was carried out according to the method describe by Kim et al., (2010) with some modifications. Porcine pancreatic lipase (PPL, type II) stock solution (2.5 mg/ml) was prepared in 0.1 M Tris HCl buffer with 5 mM CaCl₂ (pH 7.0). Reaction volume of 200 µl, containing 30 µl of 2.5 mg/ml enzyme and 120 µl of different concentrations of bark extracts (37.5, 75, 150, 300, 600 µg/ml; n = 3) were pre-incubated at 37°C for 15 min. Reaction was then started by addition of 5 µl of 10 mM p-NPB in dimethylformamide and was allowed to proceed at 37°C for 30 min. Lipase inhibitory activity of bark extracts were determined by measuring the hydrolysis of p-NPB to p-nitrophenol at 405 nm using SpectraMax384micro plate reader. Inhibition of lipase activity was expressed as the percentage decrease in optical density when pancreatic lipase was incubated with bark extracts. Lipase inhibition (%) was calculated according to the following formula;

\[
\text{Inhibition} \% = \frac{(A-a) - (B-b)}{(A-a)}
\]

Where, A is the activity without inhibitor, a- the negative control without inhibitor, B- the activity with inhibitor and b is the negative control with inhibitor.

E. Cholesterol esterase inhibitory activity of Ceylon cinnamon

Pancreatic cholesterol esterase inhibitory activity of bark extracts of Ceylon cinnamon were performed according to the method described by Pietch & Gutschow, (2005) with some modifications. Reaction volume of 200 µl, containing different concentrations of bark extracts (3.12, 6.25, 12.5, 25, 50, 100µg/ml; n=3) were pre-incubated with 50 µl of 24 mM taurocholic acid and 5 µl of 8 mM p-NPB in acetonitrile in 0.1M sodium phosphate buffer containing 0.1M NaCl (pH 7.0) at 25°C for 10 min. Reaction was then initiated by addition of 42.5 µl of (1.25 µg/ml) cholesterol esterase enzyme and it was
monitored at 25°C for 6 min at 405 nm using SpectraMax384 micro plate reader. The IC₅₀ values were calculated from the linear steady state turnover of the substrate.

F. Cholesterol micellization inhibitory activity of Ceylon cinnamon

Artificial micelles were used as a model system for in vitro cholesterol solubilization, which contains predominantly uniform particles based on sodium taurocholate, egg lecithins, cholesterol and oleic acid to reflect the natural mixed micelle. Further, they were prepared according to the method described by Kirana et al., (2005) with some modifications. Briefly, the solution containing 2 mM cholesterol, 1 mM oleic acid and 2.4 mM phosphatidylcholine were dissolved in methanol. Then these samples were dried under nitrogen before adding 15 mM phosphate-buffered saline (PBS) containing 6.6 mM taurocholate salt, pH 7.4. The suspension was sonicated twice for 30 min using a sonicator (Bandelin SANOREX electronic, RK 510) and incubated overnight at 37 °C. Different concentration of ethanol and DCM:M bark extracts (0.25, 0.5 and 1.0 mg/ml; n=6) and were added to the mixed micelle solution and were incubated at 37 °C for further 2 h. The solution was centrifuged at 16,000 rpm for 20 min. The supernatant was collected and cholesterol concentration was determined using total cholesterol test kit (BXC0261, Fortress diagnostics, UK). PBS was used as the control and EGCG as the positive control in the assay.

G. Statistical analysis

Data represented as mean ± SD. Data of each experiment were statistically analysed using SAS version 6.12. One way analysis of variance (ANOVA) and the Duncan’s Multiple Range Test (DMRT) were used to determine the differences among treatment means. P < 0.05 was regarded as significant.

III. RESULTS

A. Anti-lipase activity of Ceylon cinnamon

Both ethanol and DCM:M bark extracts of Ceylon cinnamon showed moderate anti-lipase activity and anti-lipase activity among ethanol and DCM:M bark extracts were statistically insignificant (p < 0.05). The IC₅₀ values of ethanol bark and DCM:M bark extracts were 301.09 ± 5.73 and 297.57 ± 11.78 µg/ml respectively. The dose response relationship of ethanol bark and DCM:M bark extracts is given in Table 1.

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanol bark</td>
</tr>
<tr>
<td>37.5</td>
<td>55.27 ± 3.59</td>
</tr>
<tr>
<td>75</td>
<td>49.54 ± 0.29</td>
</tr>
<tr>
<td>150</td>
<td>27.35 ± 4.43</td>
</tr>
<tr>
<td>300</td>
<td>5.30 ± 1.28</td>
</tr>
<tr>
<td>600</td>
<td>5.74 ± 0.80</td>
</tr>
<tr>
<td>IC₅₀</td>
<td>301.09 ± 5.73</td>
</tr>
</tbody>
</table>

Data represented as mean ± SD (n=3). IC₅₀ values in the columns superscripted by different letters were significantly different at p < 0.05.

B. Cholesterol esterase inhibitory activity of bark extracts of Ceylon cinnamon

Cholesterol esterase inhibitory activity of bark extracts of Ceylon cinnamon is given in Fig 1. Ceylon cinnamon possesses significant (p < 0.05) in vitro cholesterol esterase inhibitory activity in a dose dependent manner. However, ethanol bark had significantly high activity compared to DCM:M bark extract (p < 0.05). The IC₅₀ values of ethanol bark and DCM:M bark extracts were 30.62 ± 1.67 and 34.39 ± 0.91 µg/ml respectively.

C. Cholesterol micellization inhibitory activity of bark extracts of Ceylon cinnamon

Both ethanol and DCM:M bark extracts of Ceylon cinnamon showed Cholesterol micellization inhibitory activity in a dose dependent manner. However, ethanol bark extract had significantly high (p < 0.05) activity compared to DCM bark extract. The IC₅₀ values of ethanol bark and DCM:M bark extracts were 0.23 ± 0.02 and 0.48 ± 0.02 mg/ml respectively. The dose response relationship of ethanol bark and DCM:M bark extracts for Cholesterol micellization inhibitory activity is given in Table 2.
Figure 1:

Fig 1. Cholesterol esterase inhibitory activity of ethanol and DCM:M bark extracts of Ceylon cinnamon. IC\textsubscript{50} values: Ethanol bark 30.62 ± 1.67 µg/ml; DCM:M bark: 34.39 ± 0.91 µg/ml. IC\textsubscript{50} values superscripted by different letters are significantly different at p < 0.05.

Table 2. Cholesterol micellization inhibitory activity of bark extracts of Ceylon cinnamon

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>% Inhibition of cholesterol solubility in micelles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanol bark</td>
</tr>
<tr>
<td>0.25</td>
<td>98.09 ± 1.25</td>
</tr>
<tr>
<td>0.5</td>
<td>69.48 ± 1.99</td>
</tr>
<tr>
<td>1</td>
<td>49.48 ± 1.90</td>
</tr>
<tr>
<td>IC\textsubscript{50}</td>
<td>0.23 ± 0.02\textsuperscript{a}</td>
</tr>
</tbody>
</table>

Data represented as mean ± SD (n=6). IC\textsubscript{50} values in columns superscripted by different letters are significantly different at p < 0.05.

IV. DISCUSSION

Hyperlipidaemia is a group of metabolic disorders characterized by elevated levels of serum triglycerides and cholesterol (Jacobson et al., 2007). It plays an important role in pathogenesis of obesity, diabetes, hypertension and cardiovascular diseases (Fauci et al., 2008). The prevalence and incidence of hyperlipidaemia is increasing rapidly throughout the world due to variety of factors. These factors include sedentary life styles, lack of physical exercise, stress and consumption of high fat diets (Fauci et al., 2008). Currently, available antilipidaemic drugs are expensive and induce undesirable side effects (Fauci et al., 2008). In this regard many natural products have been evaluated for antilipidaemic properties and can be used in prevention and management of hyperlipidaemia (Adisakwattana et al., 2012; Kumar et al., 2011; Uahiyama et al., 2011; Ikeda et al., 2010).

In this study we evaluated the antilipidaemic properties of bark extracts of Ceylon cinnamon via anti-lipase activity, cholesterol esterase and cholesterol micellization inhibitory activities. Ceylon cinnamon bark possesses all the antilipidaemic properties tested in this study. The cholesterol esterase and cholesterol micellization inhibitory activities showed potent activities whereas lipase inhibitory activity was moderate (Orlistat IC\textsubscript{50} = 0.35 µl/ml, Bustanji et al; 2010). Some studies have shown that cinnamon has antilipidaemic properties via HMG-CoA reductase inhibitory activity (Lee et al., 2003) and through activation of peroxisome proliferator-activated receptors mechanisms (Sheng et al., 2008). Recently antilipidaemic properties of Ceylon cinnamon have shown in a rat study by Ranasinghe et al., (2012). An inhibition of fat absorption and suppression of lipid absorption can be mediated by three main mechanisms: inhibition of pancreatic cholesterol esterase activity (Kumar et al., 2011); impairment of cholesterol micellization (Vermeer et al., 2013); and inhibition of bile acid binding (Adisakwattana et al., 2012). However, inhibition of fat digestion and absorption and thereby suppression of lipid digestion and absorption via anti-lipase activity, cholesterol esterase and cholesterol micellization inhibitory activities of Ceylon cinnamon were not previously reported. Therefore, these novel antilipidaemic properties add values to Ceylon cinnamon as a natural good source with antilipidaemic activity via multiple mechanisms. As Ceylon cinnamon is called as true cinnamon worldwide and currently Sri Lanka produces > 90 % of the world’s production these novel findings with therapeutic value may be useful in value addition to Ceylon cinnamon in the international trade. Further, consumption of bark of Ceylon cinnamon in daily life may be important for prevention and management of hyperlipidaemia and related chronic diseases. Moreover, there is a possibility to isolate active compounds from bark of...
Ceylon cinnamon with anti-cholesterol esterase activity and cholesterol micellization inhibitory activities for development of functional foods, nutraceuticals, cosmeaceuticals and pharmaceuticals for prevention and management of hyperlipidaemia and related chronic diseases.

We have previously reported anti-oxidant properties of bark extracts of Ceylon cinnamon (Abeysekera et al., 2013). Bark extracts of Ceylon cinnamon had potent anti-oxidant activities via multiple mechanisms (Abeysekera et al., 2013). Oxidative stress is now known to be involved in hyperlipidaemia; it is indeed an early event in the evolution of hyperlipidaemia (Jin et al., 2013). As free radicals are involved in lipid peroxidation and related hyperlipidaemic activities anti-oxidants can play a vital role in antilipidaemic activities. It has been reported that phenolic compounds show the ability to inhibit the formation of cholesterol micelles (Vermeer et al., 2013). Therefore, observed antilipidaemic activities of bark extracts of Ceylon cinnamon may be due to the presence of anti-oxidative compounds. Further, experiments are in progress to isolate active compounds and efficacy in vivo studies.

V. CONCLUSIONS

It is concluded that bark extracts of Ceylon cinnamon possess lipase, cholesterol esterase and cholesterol micellization inhibitory activities. This is the first study to report anti-lipase, cholesterol esterase and cholesterol micellization inhibitory activities of Ceylon cinnamon worldwide.

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Acetylcholinesterase Inhibitory and Antioxidant Activities of Caesalpinia Bonduc L. Bark

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Abstract—The growth in the aging population has increased the number of patients with Alzheimer’s disease (AD) worldwide. The naturally occurring enzyme inhibitors and antioxidants play an important role in a drug discovery program for such diseases. Caesalpinia bonduc L. (Fabaceae) is a medicinal plant used widely in the traditional system of medicine in the Asian region of the world. In the present study, the total ethanolic extract of bark of C. bonduc was evaluated for Acetylcholinesterase (AChE) enzyme inhibitory activity and antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging, ferrous iron chelating (FICA) and ferric reducing antioxidant potential (FRAP). Ethanolic extract of bark of C. bonduc exhibited moderate AChE inhibitory activity with an IC50 value of 190.76 ± 2.8 μg/mL while the IC50 of the Galanthamine, a clinically used inhibitor was 0.46 μg/mL. Bark extract showed good DPPH radical scavenging activity with an IC50 value of 83.69 ± 0.1 μg/mL, in comparison with that of Trolox (IC50 = 4.50 ± 0.3 μg/mL). Lower ferrous ion chelating effect was detected for the bark extract with an IC50 value of 3450.6 ± 235.19 μg/mL with comparison to that of EDTA (12.74 ± 0.2 μg/mL). The FRAP assay resulted the mg Trolox equivalent/g of extract of C. bonduc as 233.6 ± 0.2 mg. The results indicated that the ethanol extract of bark of C. bonduc showed AChE inhibitory, DPPH radical scavenging, FICA and FRAP activity. Therefore the in vitro assay data indicates the potential of the extract for further AChE inhibitory and antioxidant bioactive studies including activity-guided fraction of bioactive compounds.

Keywords—Caesalpinia bonduc, acetylcholinesterase, antioxidant

I. INTRODUCTION

Neurodegenerative diseases such as Alzheimer’s disease (AD) typically begin with subtle recognition failure and memory lapses. It slowly becomes more severe and eventually, incapacitates the individual’s mental abilities. The drugs available for AD function by increasing the acetylcholine levels in the brain, which in turn enhances the signal transfer at the synapses (Ferreira et al., 2006). Therefore cholinesterase enzyme (ChE) inhibitors are among the drugs most widely used in the treatment of AD. Currently drugs such as donepezil, galanthamine together with antioxidants derived from herbal extracts such as from Gigngo biloba are being used in the clinical practice for AD treatment (Orhan et al., 2006).

Lead compounds of many western drugs have originated from bioactive plant extracts. Owing to this factor in recent times there is a growing focus on plant-based research worldwide. Furthermore medicinal plants have been known as sources of therapeutics for thousands of years.

Caesalpinia bonduc L. (Fabaceae) is a medicinal plant widely distributed in the tropical regions of Asia and the Caribbean. The plant is being used in the traditional system of medicine in countries such as Sri Lanka, India, Nicobar Islands (Singh and Raghav, 2012). In Sri Lanka it is commonly called as “Kumburu”. Pharmacological studies of the seeds and leaves have reported antioxidant, anti-inflammatory, antimalarial, antimicrobial, antidiarrheal, antidiabetic, antitumor, antihelmintic, antifilarial, hepatoprotective, antirheumatic and antipyretic activities (Singh and Raghav, 2012). Ata et al., (2009) has recorded anti glutathione S-transferase assay guided isolation of a sterol namely 17–hydroxy-campesta-4, 6-dien-3-one from the ethanolic bark extract of C. bonduc. Previous phytochemical studies have also reported the isolation of diterpenoids such as neocaselpin H, cordylane A, caesalpinin B, bonducellpin E, caesalpinolide A, 17-methylouacapane8 (14), -9(11)-diene and neocasalpin P, homoisoflavonoids namely, caesalpinianone, 6’-O-methylcaesalpinianone and other compounds such
as, hematoxylin, stereocheno1, 4,6'-O-acetylloganic acid, 4'-O-acetylloganic acid and 2-O-ß-D-glucosylxy-4-methoxybenzenepropanoic acid from the ethanolic extract of bark (Ata et al., 2009; Ata et al., 2009; Udenigwe et al., 2007).

The present study was undertaken to evaluate the acetylcholinesterase inhibitory activity of the ethanol extract of bark of C. bonduc and its ability to function as an antioxidant.

II. MATERIAL AND METHODS

A. Collection, preparation and extraction of plant material

Bark of C. bonduc was collected from Chilaw, Sri Lanka. The voucher specimen of C. bonduc was deposited at the Herbal Technology Section at the Industrial Technology Institute, Sri Lanka. The collected bark was shade-dried. The 100 grams of the powdered bark was extracted with ethanol (250 mL) using cold extraction technique (Wu et al., 2003). The plant material was extracted three times with ethanol. The filtrates were combined and concentrated to dryness under vacuum using a rotary evaporator to obtain the crude extract.

B. Acetylcholinesterase inhibitory activity

Acetylcholinesterase (AChE) inhibition was determined using a modified method of Ellman et al., (1961). A total reaction volume of 200 µl containing 0.002 U/mL of AChE (10 µl), different concentrations of ethanolic extracts and 0.1 M sodium phosphate buffer (pH 8.0), was pre incubated for 15 min at 25 °C. The reaction was then initiated by the addition of 0.71 mM acetylthiocholine and 0.5 mM DNTB in 20 µl of 0.1 M sodium phosphate buffer. The hydrolysis of acetylthiocholine was monitored by the formation of yellow colour 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocoheline for a period of 10 min at λ = 412 nm. Galanthamine was used as the positive control. The kinetic parameter Vmax was used to calculate the % inhibition and IC50 value (1).

\[ \% = \frac{[V_{\text{max}} \ (\text{Control}) - V_{\text{max}} \ (\text{Test})]}{V_{\text{max}} \ (\text{Control})} \times 100 \quad \text{Inhibition} \quad (1) \]

C. Antioxidant activity

1) 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity: Free radical scavenging activity was measured by the Blois (1958), method with some modifications. The reaction mixture contained different concentrations of the ethanol extract and 200 µl of 40 μg/ml solution of DPPH in methanol. Reaction volume was made up to 300 µl by using analytical grade methanol. The mixture was left to stand for 10 minutes in the dark. The absorption was measured at λ = 517 nm against a corresponding blank (methanol). Trolox was used as the positive standard. The capability to scavenge the DPPH radical by 50% (IC50) was calculated using the equation (2).

\[ \% = \frac{[\text{Abs (Control)} - \text{Abs (Test)}]}{\text{Abs (Control)}} \times 100 \quad \text{Inhibition} \quad (2) \]

2) Ferrous iron chelating activity: Metal ion-chelating effect of the bark extract for ferrous ions was measured according to the method by Carter (1971) with some modifications. Reaction mixture of 200 µl containing distilled water, 20 µl of Ferrous sulphate, different concentrations of the ethanolic bark extract and 40 µl of Ferrozine in distilled water was added and the plate was incubated at room temperature for 10 minutes. The absorbance was measured at λ = 562 nm. EDTA was used as the positive standard. Percentage chelating effect was calculated using the following equation (3) and IC50 value was calculated.

\[ \% = \frac{[\text{Abs (Control)} - \text{Abs (Test)}]}{\text{Abs (Control)}} \times 100 \quad \text{Chelation} \quad (3) \]

3) Ferric reducing antioxidant activity (FRAP): A modified protocol of Benzie and Szeto’s (1999) was adopted for the FRAP assay. The fresh working solution of FRAP was prepared by mixing 25 mL acetate buffer, 2.5 mL of 2, 4, 6-tripryidyl-striazine (TPTZ) and 2.5 mL of FeCl3.6H2O. Different concentrations of the ethanol extract of bark was allowed to react with 150 µl of FRAP solution. The plate was vortexed and left to stand for 8 minutes. Absorbance of the Ferrous tripryidylstriazine complex was measured at λ = 593 nm. The standard curve was linear between 10 μg/mL and 120 μg/mL Trolox. Results are expressed in mg Trolox equivalent/g of extract using the standard curve of Trolox.

III. STATISTICAL ANALYSIS

All experiments were performed in triplicates using a 96 well micro plate reader Spectra Max 340 (molecular devices, CA, USA). The results are
presented as in mean ± Standard Error (SE). The IC<sub>50</sub> values were calculated by linear regression analysis. Results were calculated by employing the Soft Max Pro program and Microsoft Office Excel for Mac 2008.

IV. RESULTS AND DISCUSSION

Pharmacological studies have discovered a vast range of bioactivities of phytochemicals, which has lead to a growing interest in the exploitation of plants for their therapeutic principles. Oxidative stress is known to be a causative agent for the development of degenerative diseases such as AD, cancer and cardiovascular diseases (Ames et al., 1993). In the present study <i>C. bonduc</i> was evaluated for the first time for its AChE inhibitory activity along with antioxidant activity <i>in vitro</i>.

A. AChE inhibitory activity

The IC<sub>50</sub> values obtained for the ethanol extract of bark of <i>C. bonduc</i> and the standard Galanthamine are given in Table 1. The inhibitory activity of different concentrations is summarized in Figure 1. In comparison with the standard, Galanthamine (IC<sub>50</sub> 0.46 ± 0.02 μg/mL), the bark extract exhibited moderate <i>in-vitro</i> acetylcholinesterase enzyme inhibitory activity.

Table 1. AChE inhibitory activity of ethanolic extract of <i>C. bonduc</i> bark

<table>
<thead>
<tr>
<th>Plant/ Compound</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;i&gt;C. bonduc&lt;/i&gt;</td>
<td>190.76 ± 2.8</td>
</tr>
<tr>
<td>Galanthamine</td>
<td>0.46 ± 0.02</td>
</tr>
</tbody>
</table>

N=3  Data represented as mean ± SE

B. Antioxidant activity

The anti oxidant activity of the ethanolic bark extract was evaluated by three methods: DPPH, FRAP and FICA. Results for all three assays are presented in Table 2.

1) DPPH radical scavenging activity: The DPPH test intends to measure the capacity of the extract to scavenge the DPPH free radical by donating a hydrogen atom or electron in solution (Tepe et al., 2005). The anti-oxidant concentration required for 50% radical scavenging per unit DPPH exerted by the ethanolic bark extract of <i>C. bonduc</i> and positive standard Trolox is summarized in Table 2. The DPPH radical scavenging activity increased with the increasing of the sample concentration. The IC<sub>50</sub> value of the extract was 83.69 ± 0.1 μg/mL in comparison to that of Trolox (IC<sub>50</sub> 4.50 ± 0.3 μg/mL) which is a known antioxidant.

Table 2. Antioxidant activity of ethanolic extract of <i>C. bonduc</i> bark

<table>
<thead>
<tr>
<th>Plant/ Compound</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (μg/mL)</th>
<th>mg TE/g of extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPH</td>
<td>83.69 ± 0.14</td>
<td></td>
</tr>
<tr>
<td>FICA</td>
<td>3450.6 ± 235.19</td>
<td></td>
</tr>
<tr>
<td>FRAP</td>
<td>233.6 ± 0.14</td>
<td></td>
</tr>
<tr>
<td>C. bonduc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trolox</td>
<td>4.50 ± 0.0</td>
<td>-</td>
</tr>
<tr>
<td>EDTA</td>
<td></td>
<td>12.74 ± 0.2</td>
</tr>
</tbody>
</table>

N=3  Data represented as mean ± SE

2) FICA assay: Transition metals catalyse oxidation reactions and therefore in the presence of chelating agents, complex formation with these transition metals are disrupted (Gordon, 1990). Through FICA assay the antioxidant activity of a plant extract is measured by how effectively the chelating compounds in it can compete with ferrozine for ferrous ion. The presence of chelating compounds in the extracts can disrupt the formation of ferrozine-Fe<sup>2+</sup> complex. Ion chelating capacity of the ethanolic bark extract and the metal chelator EDTA were evaluated and the values are given in Table 2. The IC<sub>50</sub> value of the ethanolic bark extract was found to be 3450.6 ± 235.19 μg/mL where as the IC<sub>50</sub> value of standard EDTA was observed as 12.74 ± 0.2 μg/mL. Therefore this indicates that the ethanolic bark extract of <i>C. bonduc</i> is a very weak chelator of iron (II) ions.

3) FRAP assay: The reducing capacity of a compound serves as a significant indicator of its potential antioxidant activity. The mg Trolox equivalent/g of extract of <i>C. bonduc</i> was found to be 233.6 ± 0.2 mg (Table 2).
Broad spectrum of compound classes such as alkaloids, tannins, terpenoids and flavonoids are known to exhibit AChE inhibitory and antioxidant activities (Adewusi et al., 2011). Therefore previously isolated diterpenoids (Ata et al., 2009), flavonoids (Ata et al., 2009) and sterols (Udenigwe et al., 2007) from the bark of C. bonduc may account for the observed AChE inhibitory and antioxidant activity in the present study. Hence, further studies are required to identify the acetylcholinesterase inhibitory and antioxidant active compounds from the ethanolic bark extract.

V. CONCLUSIONS

In summary, the crude ethanolic bark extract of C. bonduc possess moderate levels of AChE inhibitory activity and antioxidant activity. Plant extract should be further subjected to bioassay guided isolation of compounds by chromatographic techniques to identify the potential chemical entities for therapeutic use in the treatment of AD.

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Evaluation of the Effect of the Water Extract of Dried Flowers of Aegle marmelos on Na+/K+ ATPase Activity in Liver, Erythrocytes and Small Intestine of Diabetic Rats

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Abstract - The water extract of Aegle marmelos (Beli mal Drink) is a popular beverage used by Sri Lankans with many medicinal benefits and the hypoglycaemic and anti-inflammatory effects of this beverage have been scientifically validated. Under many pathological conditions alterations in Na⁺/K⁺ ATPase activity in tissues are observed and these may lead to many metabolic changes during diseases. The present study evaluated the effect of the water extract of dried flowers of Aegle marmelos on Na⁺/K⁺ ATPase activity in liver, erythrocytes and small intestine of diabetic Wistar rats. Experimental diabetes mellitus was induced in rats by the intra-venous administration of Alloxan monohydrate (40 mg/kg) and rats with a serum glucose concentration of > 7.00 mmol/L were selected for the experiments. Normal and diabetic control groups received distilled water whereas the Diabetic test group received a 500 mg/kg dose of test extract. After ½ h, each rat was euthanized to collect blood samples as well as the livers and small intestines. The Na⁺/K⁺ ATPase activity in membrane preparations of liver, erythrocytes and small intestine of diabetic Wistar rats. Experimental diabetes mellitus was induced in rats by the intra-venous administration of Alloxan monohydrate (40 mg/kg) and rats with a serum glucose concentration of > 7.00 mmol/L were selected for the experiments. Normal and diabetic control groups received distilled water whereas the Diabetic test group received a 500 mg/kg dose of test extract. After ½ h, each rat was euthanized to collect blood samples as well as the livers and small intestines. The Na⁺/K⁺ ATPase activity in membrane preparations of liver, erythrocytes and small intestine, was determined in terms of liberation of inorganic phosphate during enzymatic ATP hydrolysis. The Na⁺/K⁺ ATPase activity in the liver plasma membrane was reduced significantly (P < 0.001) by 49.4 % in diabetic rats compared to normal rats. It was increased significantly (P < 0.001) by 44.3 % in diabetic rats after administration of the test extract compared to Diabetic control rats. The treatment with the test extract caused a significant increase in Na⁺/K⁺ ATPase activity in all three tissues compared to Diabetic control rats and this showed the test extract has an acute effect on the Na⁺/K⁺ ATPase activity in tissues of diabetic rats. The water extract of dried flowers of Aegle marmelos is a herbal beverage which can be used to revise the short term complications of diabetes associated with impaired Na⁺/K⁺ ATPase activity in tissues.

Keywords: Na⁺/K⁺ ATPase activity, Diabetes, Aegle marmelos

I. INTRODUCTION

The Na⁺/K⁺ ATPase is a plasma membrane bound enzyme complex that plays a fundamental role in cellular functions by maintaining the electrochemical gradient of Na⁺ and K⁺ ions between two sides of the plasma membrane. It is categorized under the P-type ATPase family of cation pumps that use the free energy of hydrolysis of high energy phosphate bond of ATP to actively transport cations against their electro chemical gradients. The Na⁺/K⁺ ATPase is an integral protein which transports 3Na⁺ ions outside and simultaneously 2K⁺ ions inside across the cell membrane. Thus it creates an ion gradient which is associated with the Na-coupled transport of nutrients into cells, osmotic balance, cell volume regulation and maintenance and restoration of the resting membrane potential in excitable cells (Koksoy, 2002).
The Na\(^+\)/K\(^+\) ATPase activity in the plasma membrane is altered in many pathological conditions, such as diabetes, cardiac diseases, hypertension, some cancers, etc. The individuals and animals with diabetes have been reported to express changes in Na\(^+\)/K\(^+\) ATPase activity in many tissues such as heart, peripheral nerve, kidney, liver, erythrocytes and intestine. The impairment of the Na\(^+\)/K\(^+\) ATPase activity may play a role in the development of the chronic complications of diabetes such as retinopathy, nephropathy, neuropathy and premature vascular disease. The intensity and direction of the alterations depend on the duration of diabetes and the tissue involved (Jaitovich and Bertorello, 2006).

*Aegle marmelos* correa (L) which belongs to family Rutacea, is commonly known as bael fruit and a plant used extensively in traditional medicinal systems of Asian counties. The literature revealed that various parts of this plant have been used to treat complications of diabetes mellitus over centuries (Sharma et al., 2007). A beverage that is prepared by boiling the dried flowers in water is popular among Sri Lankans as it has a refreshing, soothing and calming effect. The hypoglycaemic and anti-inflammatory effect of this beverage is established in rats and humans (Kumari et al., 2013; Kumari et al., 2014). The present study is designed to evaluate the effect of the water extract of dried flowers of AM on the Na\(^+\)/K\(^+\) ATPase activity in diabetic Wistar rats.

**II. METHODOLOGY**

**Ethical approval**
Ethical approval was taken from the Ethics Review Committee of University of Sri Jayewardenepura, Sri Lanka. (Ref no: 432/09). International guidelines and recommendations of Federation of European Laboratory Animal Science Associations (FELASA) were followed for handling of animals. Assays were carried out at the Animal House and the Department of Biochemistry of University of Sri Jayewardenepura, Sri Lanka.

**Plant material**
Dried flowers were purchased from farmers from different provinces of the country and pooled together. Required amounts were taken from this pool for the preparation of extracts. The plant material was authenticated by the National Herbarium, Peradeniya.

**Preparation of the extract**
The water extract of dried flowers of *Aegle marmelos* (WEAM) was prepared by boiling 25 g of dried flowers in 500ml of water and reduced to 50 ml. The freshly prepared extract was used for experiments.

**Animals**
Healthy adult male, Wistar rats weighing 150 - 200 g were purchased from Medical Research Institute, Colombo 8, Sri Lanka. Rats were housed under standard conditions (230 ± 2 0C, 60 % - 70 % relative humidity and 12 h photo period) and fed with standard diet and water *ad libitum*.

**Induction of Diabetes**
Experimental Diabetes mellitus was induced in rats by the intra-venous administration of 40 mg/kg of Alloxan monohydrate. Fasting blood glucose levels were determined after 72 h and rats with a serum glucose concentration of > 7.00 mmol/L were used for the experiment.

**Evaluation of the Na\(^+\)/K\(^+\) ATPase activity in diabetic rats**
Diabetic rats were randomly categorized in to two groups as the Test and Control groups and another six rats were selected for the Normal control group from the rats that belonged to the same batch but not treated with alloxan. After one week of diabetes induction, the following were administered to the rats using oral feeding needles. Normal control group and Diabetic control group received 2.5 ml of distilled water while the Diabetic test group received a 500 mg/kg dose of WEAM. After 1/2 h each rat was euthanized and a blood sample was collected by a ventricular puncture and the liver and small intestine were removed.

**Liver plasma membrane preparation**
The removed liver was immediately washed with ice cold saline and frozen in liquid nitrogen and stored at -80 C until use. Liver plasma membranes were prepared by the method described by Askari *et al.*, (1997) and the whole procedure was carried out at 4 C. Frozen rat liver samples (1 g) were homogenized (Ultra- Turrax T-25) in 5 ml of ice-cold 1 mM NaHCO\(_3\) ([pH 7.5] (Himedia, India)) and the homogenates were diluted and filtered through 3 layers of surgical gauze. These were centrifuged (Vision Scientific, VS-550) at 1500 g for 10 min at 4 C and the pellets were re-suspended in 1 ml of ice-cold buffer (1 mM NaHCO\(_3\), pH 7.5). Then 5.5
volume of 70.7 % sucrose were added and mixed. After transferring into a 50 ml centrifuge tube, 8 ml of 48.2 % and 4 ml of 42.5 % sucrose ([Sigma Aldrich, USA]) were added. Following centrifugation for 30 min at 19 000 g, the substance collected at the interface of two layers of sucrose ([42.5 % and 48.2 %], [Sigma Aldrich, USA]) was drained and washed. The final pellet was re-suspended in 0.25 M sucrose, 30 mM histidine (Sigma Aldrich, USA), 1 mM EDTA ([pH 6.8] [Sigma Aldrich, USA]), and stored at −80 °C. These liver plasma membrane preparations were enriched with Na+/K+ ATPase. Total protein estimation was done for unification using total protein kit (Sigma TP0 300) and the test solution was prepared to a final concentration of 1 mg of protein per ml.

The mucosal preparations of small intestines

The mucosal preparations of small intestines were prepared by the method described by Barada et al., (1994) and the whole procedure was carried out at 4 °C. Removed small intestines were flushed with ice-cold 2 M NaCl (Himedia, India) and cut along the anti-mesenteric border. The mucosae were scraped into 5 ml of ice cold 2 M NaCl and centrifuged at 6800 g for 15 min at 4 °C and washed three times with ice-cold 50 mM Tris, 5 mM EDTA (pH 7.2) and two times with 50 mM Tris, 1 mM EDTA ([pH 7.2], [Sigma Aldrich, USA]) and homogenized at 4 °C in 250 mM sucrose, 30 mM histidine and 1 mM EDTA buffer (pH 7.2). Total protein estimation was done for unification and the test solution was prepared to a final concentration of 1 mg of protein per ml.

Erythrocyte membrane preparation

Blood samples were collected in EDTA tubes and plasma was removed to store at −80 °C for further assays. Erythrocyte membranes were isolated according to the method described by Kassak et al., (2006) with some modifications. The whole procedure was carried out at 4 0C and whole blood samples were separated by centrifugation at 900 g for 10 min at 4 °C. Excess plasma and platelets were discarded and packed erythrocytes were washed three times with ice cold PBS (10 mM phosphate buffer, 150 mM NaCl, pH 7.4). The pellet was re-suspended in 1 ml of ice cold PBS and five volumes of 20 mM Tris-EDTA-HCl buffer (pH 7.4) was added to initiate haemolysis. Following centrifugation at 19 500 g for 10 min at 4 °C, double washing with 20 mM, 10 mM and 5 mM Tris-EDTA-HCl buffer was done. For unification of the suspensions, protein concentration was estimated using a total protein kit and the ghosts were re-suspended to a final concentration of 1 mg of protein per ml in 5 mM Tris-EDTA-HCl buffer (pH 7.4).

Measurement of Na+/K+ ATPase activity

Measurement of the Na+/K+ ATPase activity was carried out as previously described by Kassak et al., (2006) and it was determined in terms of liberation of inorganic phosphate during enzymatic ATP hydrolysis. Standard curve was drawn for liberation of inorganic phosphate by various concentrations of KH2PO4 (8 x 10⁻⁵ mol/L – 52 x 10⁻⁵ mol/L). The reaction medium was prepared using 100 mM Tris-HCl, 10 mM MgCl2 (Himedia, India), 15 mM KCl (Himedia, India), 85 mM NaCl, 1 mM EDTA and 2 mM ATP ([Sigma Aldrich, USA] (pH 7.4)). To observe total ATP hydrolysis 15 μl of ghost suspension was incubated with 55 μl of medium at 37 °C for 30 min. As a control for non-enzymatic hydrolysis of ATP another 15 μl of the ghost suspension from the same samples were mixed with 55 μl of medium and incubated at 4 °C for 30 min. To determine the Mg²⁺ATPase activity, the mixture was incubated in the presence of 0.2 mM Ouabain (Sigma Aldrich, USA), which inhibits the Na+/K+ pump. An equal volume of 15 % Trichloroacetic acid (LOBA Chemie, India) was added to stop the reactions. Inorganic phosphate liberated during ATP hydrolysis formed a colored product by the reaction between ammonium molybdate (Sigma Aldrich, USA) and amino naphospholic acid ([ANS] [Sigma Aldrich, USA]) and absorbance values were estimated spectrophotometrically at 640 nm (Uluwaduge, 2002). The Na+/K+ ATPase activity (Na+/K+ AA) was evaluated by following equation,

\[
\text{Na+/K+ AA} = \text{Total ATPase activity} - (\text{Mg}^{2+} - \text{ATPase activity} + \text{non enzymatic hydrolysis of ATP})
\]

III. RESULTS

The mean Na+/K+ ATPase concentration of hepatocyte plasma membranes in Normal control, Diabetic control and Diabetic test groups were 5.04 ± 3.9, 2.55 ± 1.5 and 4.58 ± 3.8 nmol per mg of protein respectively. The Na+/K+ ATPase activity in the liver plasma membrane was reduced significantly (P <0.001) by 49.4 % in diabetic rats compared to normal rats. It was increased significantly (P <0.001) by 44.3 % in diabetic rats after administration of the test extract compared to Diabetic control rats (Figure 1).
The mean Na\(^+\)/K\(^-\) ATPase concentration of mucosae of small intestine in Normal control, Diabetic control and Diabetic test groups were 1.40 ± 2.6, 2.4 ± 1.6 and 3.2 ± 2.1 nmol per mg of protein respectively. The Na\(^+\)/K\(^-\) ATPase activity in the small intestine was increased significantly (P <0.01) by 41.5 % in diabetic rats compared to normal rats. It was further increased significantly (P <0.01) by 24.4 % in diabetic rats after administration of the test extract compared to Diabetic control rats (Figure 1).

The mean Na\(^+\)/K\(^-\) ATPase concentration of erythrocyte membranes in Normal control, Diabetic control and Diabetic test groups were 2.93 ± 4.4, 2.07 ± 1.5 and 3.19 ± 2.1 3 nmol per mg of protein respectively. The Na\(^+\)/K\(^-\) ATPase activity in the erythrocytes was reduced significantly (P <0.001) by 29.3 % in diabetic rats compared to normal rats. It was increased significantly (P <0.001) by 35.2 % in diabetic rats after administration of the test extract compared to Diabetic control rats (Figure 1).

**Figure 1**: The mean Na\(^+\)/K\(^-\) ATPase concentration of erythrocyte membranes, liver plasma membranes and mucosae of small intestines of the rats in normal control (N. control), diabetic control (D. control) and diabetic test groups.

![Graph showing Na\(^+\)/K\(^-\) ATPase activity in different tissues.](image)

**IV. DISCUSSION**

The enzyme Na\(^+\)/K\(^-\) ATPase is critical for the maintenance of the electrochemical gradient across the plasma membrane and is associated with regulation of the fundamental functions of the cell (Koksoy, 2002). Scientific investigations have reported that there are significant alterations in Na\(^+\)/K\(^-\) ATPase activity in tissues under many pathological conditions and these alterations observed in diabetic subjects may leads to many metabolic changes and play a role in development of diabetes associated complications (Jaitovich and Bertorello, 2006).

The previous investigations have observed the effect of diabetes on Na\(^+\)/K\(^-\) ATPase activity of various tissues is different and in some tissues diabetes induces a decrease in Na\(^+\)/K\(^-\) ATPase activity which include sciatic nerve, lens, heart, liver and erythrocyte. In some other tissues diabetes causes an increase in enzyme activity such as mucosa of the small intestine and diabetic impairment of Na\(^+\)/K\(^-\) ATPase activity could be due to altered enzyme kinetics and/or altered subunit expression (Vague et al., 2004). In the present study the Na\(^+\)/K\(^-\) ATPase activity was significantly reduced in erythrocytes and the plasma membranes of liver, while it was significantly increased in the mucosa of small intestine in diabetic rats compared to the normal rats.

Agarwal et al., (1985) also observed a reduction of the Na\(^+\)/K\(^-\) ATPase activity in the erythrocyte membrane in diabetic rats, compared to the normal rats. The diabetes-induced impairment in Na\(^+\)/K\(^-\) ATPase activity could be related to the defect in myo-inositol metabolism leading to altered lipid metabolism and lipid order in the membrane (Yorek et al., 1988). The increase in oxidative stress, the formation of advanced glycation products, the nerve growth factor metabolism (Sima and Sugimoto, 1999) and the disturbance in essential fatty acid metabolism leading to an abnormal ω6/ω3 ratio in red blood cell membrane (Djemli-Shipkolye et al., 2003) are the other metabolic changes induced by diabetes which can also downregulate the Na\(^+\)/K\(^-\) ATPase activity in erythrocytes.

A significant decrease in the Na\(^+\)/K\(^-\) ATPase activity in hepatocytes was observed by Mishra et al., (1995) in alloxan induced diabetic mice and Carnovale et al., (1991) in streptozotocin induced diabetic rats. Diabetes induced alterations in lipid composition of cell membranes cause a decrease in the fluidity of the hepatocyte membranes (Clandinin et al., 1985; Dang et al., 1989). This may lead to down regulate the Na\(^+\)/K\(^-\) ATPase catalytic a1-subunit expression in hepatocytes (Ng et al., 1993; Barada et al., 1994) and a decline in Na\(^+\)/K\(^-\) ATPase activity. The decreased enzyme activity reflect the reduced glucose transport across the cell membranes and hence leads to reduced glucose uptake by the liver during diabetes which exacerbates the hyperglycaemia.
Studies done on intestines of diabetic rats revealed that the Na⁺/K⁺ ATPase activity was increased in mucosa of the small intestine (Gnanaprapakasam and Srivastava, 1978) and mucosa basolateral membrane (Luppa and Muller, 1986) while there was no change of sodium pump activity in mucosa brush border region (Luppa and Muller, 1986). The increase in Na⁺/K⁺ ATPase activity is considered due to the up regulation of synthesis of mRNA levels of α1 and β1 isoforms (Fedorak et al., 1991; Barada et al., 1994) in mucosal cells. The increase in Na⁺/K⁺ ATPase activity in turn increases the Na⁺ dependent glucose absorption in the small intestine which leads to hyperglycaemia.

In the present study the diabetic rats were treated with the test extract and after ½ h, Na⁺/K⁺ ATPase activity was measured in erythrocytes, liver membrane and small intestine. The Na⁺/K⁺ ATPase activity in all three tissues was increased significantly compared to Diabetic control and this showed the test extract has an acute effect on the Na⁺/K⁺ ATPase activity in tissues of diabetic rats.

The test extract may act directly on the enzyme protein components or indirectly via stimulation of the secretion of insulin. According to the results of the study done on hypoglycaemic mechanisms, the test extract significantly increased the serum concentration of insulin. Insulin has a direct effect on the Na⁺/K⁺ ATPase activity and when insulin is present in high serum concentration, it increases Na⁺/K⁺ ATPase activity in tissues (Sweeney and Klip, 1998).

Previous studies have observed the direct action of insulin on isolated plasma membranes of liver and erythrocyte (Fehlmann and Freychet, 1981; Luly et al., 1981). In vitro studies done on cell membranes showed the hormone-receptor interaction could affect membrane fluidity, resulting in an alteration of the membrane microenvironment which is responsible for variations in activity of membrane-bound enzymes such as Na⁺/K⁺ ATPase (Hyslop et al., 1984). Thus the effect of elevated level of insulin may leads to the enhancement of Na⁺/K⁺ ATPase activity in diabetic rats after consumption of the test extract and the direct effect on the enzyme components should be studied further.

Insulin mediated translocation of enzyme subunits from intracellular pool to the plasma membrane is a short term regulatory mechanisms of Na⁺/K⁺ ATPase activity (Sweeney and Klip, 1998) and this may be responsible for the acute effect of the test extract on the Na⁺/K⁺ ATPase activity in diabetic rats. The results suggest the test extract is a herbal beverage which can use to revise the short term complications of diabetes associated with impaired Na⁺/K⁺ ATPase activity in tissues and further investigations should be carried out to evaluate the long term effect on the Na⁺/K⁺ ATPase activity.

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Correlation of Dengue Fever with Rainfall and Other Environmental Indices in Dehiwala Medical Officer of Health Area during 2011 & 2012

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Abstract—The present study examined the relationship between weather pattern and trend of dengue fever. It also assessed the incidence of dengue fever according to gender and Public Health Inspector (PHI) areas in 2011 and 2012 by using the notification register & the weekly return of communicable diseases (WRCD) of the Dehiwala Medical Officer of Health (MOH).

The sample consisted of 1842 confirmed cases of dengue fever. Variables were weekly confirmed dengue cases, weekly rainfall, weekly mean humidity, weekly mean temperature, PHI areas, and gender.

Findings suggested that the occurrence of confirmed dengue cases were positively correlated with, the rainfall 7 weeks preceding the registration of cases. There was no significant correlation between humidity and temperature with the confirmed dengue cases. Findings also revealed that females had higher incidence than males, and one PHI area (Badowita) reported the highest incidence for both years.

We recommend that notification data of Dengue fever be analysed at MOH level to forecast outbreaks and intensify preventive measures.

Keywords: incidence, rainfall, gender

I. INTRODUCTION

Dengue is an arboviral infection transmitted by day-biting Aedes aegypti and Aedes albopictus mosquitoes. There are four serotypes, DEN-1, DEN-2, DEN-3 and DEN-4 (Chaturvedi U C., 2008; Gunasekara M., 2009). These mosquitoes breed in small collections of water, in and around human habitats, especially in urban areas. Aedes aegypti is a day time feeder (Jacobs M., 2005). Its peak biting periods are early in the morning and in the evening before dusk. Female Aedes aegypti bites multiple times during each feeding.

Over 2.5 billion people (over 40% of the world population) are now at risk from dengue and about 50 – 100 million dengue infections occur worldwide every year. (WHO, 2013). In 2010 epidemics has been declared in the Philippines, the Caribbean, Central America and Sri Lanka (Elizabeth A.A., 2010). In Sri Lanka dengue infection was serologically confirmed in 1962 and first outbreak in 1965. First major epidemic reported in 1989 and, the disease has been endemic since 1989 with dengue haemorrhagic fever (DHF) involvement. Dengue fever (DF) has become a notifiable disease in 1996. Since year 2000 approximately 5,000 cases were reported annually (Media Seminar, 2009). During last 9 months of year 2013, 23507 suspected dengue cases have been reported from Sri Lanka. Approximately 47.64% of dengue cases were reported from the western province. The highest numbers of dengue cases in Sri Lanka were reported during the 3rd week of January 2013.

II. OBJECTIVE

General objective is to describe the epidemiological pattern of Dengue fever in Dehiwala MOH area during 2011 & 2012 based on data maintained at the MOH office.

Specific objectives of this study are to calculate incidence of DF, calculate incidence according to PHI areas in Dehiwala MOH area, and correlate
confirmed dengue cases with rainfall, wind patterns and humidity, population density according to PHI areas, housing conditions, different age groups and gender.

III. METHODS

This study is a descriptive study based on retrospective data. The study setting was Dehiwala MOH area, and covered an area of 21.09km² consists 14 PHI areas and 29 wards. The largest is Kandawala (3.05km²), and the smallest is Mount Lavinia (0.29km²).

The study was conducted according to PHI divisions such as Kohuwala, Nadimala, Saranankara, Dehiwala D1, Park, Karagampitiya, Dehiwala Central, Mount Lavinia, Mount Lavinia Central, Wedikanda, Badowita, Aththidiya, Kothalawalapura and Kandawala.

Dehiwala MOH area lying in the wet zone, receives an average annual rainfall between 2000 to 3000 mm mainly during the south west monsoon and the inter-monsoon periods. Mean temperature is around 28°C. (City profile, 2003).

The total population was estimated as 224102 in 2011 (City profile, 2003). The sample of this study is total confirmed cases of DF, DHF & Dengue Shock Syndrome (DSS) in Dehiwala MOH area during 2011 & 2012. We used tables to obtained weekly data from Dehiwala MOH Office, Meteorology Department and check list for observational data.

The following data was recorded using the notification register & the WRCD of the Dehiwala MOH office (All data collected are from year 2011 & 2012 and was collected weekly, and according to different PHI areas). No. of confirmed cases

- Out of the confirmed cases the no. of patients in different age groups (<5yrs / 6-15yrs / 16-25yrs / 26-45yrs / >45yrs).
- Out of the confirmed cases the no. of female patients & no. of male patients.
- Out of the confirmed cases the no. of patients admitted to government hospitals & no. of patients admitted to private hospitals.

Rainfall, temperature & humidity in Dehiwala MOH area during 2011 & 2012 was collected from the Meteorology Department and recorded in the tables. Data was acquired by direct observation of housing conditions, factories, hotels, schools, religious places & recreational areas in Dehiwala MOH area.

Ethical clearance for the research was obtained from the Ethics Review Committee of South Asian Institute of Technology and Medicine (SAITM) the Ethics Review no 0017/13. Administrative permission from the Provincial Director of Health Services (PDHS)-Western Province & Regional Director of Health Services (RDHS)-Colombo was obtained to get the relevant data from Dehiwala MOH office. Data was collected with minimum disturbance to the duties of the PHIs and the Supervising PHIs and the MOH. Identity of the patients was kept confidential.

The confirmed data was classified according to gender and PHI area and got total confirmed new cases per each year. Total population of Dehiwala MOH area and gender percentage in Sri Lanka was taken according to The Census Department of Sri Lanka and incidence was calculated in 2011 & 2012. Then incidence of DF calculated according to the following equation. Estimated population according to gender and PHI area were taken separately as the total population to calculate incidence according to each section.

\[
\text{Incidence} = \frac{\text{No. of new confirmed cases per year}}{\text{Total Population}} \times 1000
\]

Then we included weekly rainfall, weekly mean temperature, and weekly mean humidity in our data analysis. We assessed the relationship between weekly rainfall, weekly mean temperature, weekly mean humidity and weekly confirmed dengue cases by using SPSS software.

III. RESULTS

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Number of cases</th>
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<td></td>
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<tr>
<td>----------</td>
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<td>PHI Area</td>
<td>Total*</td>
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</tr>
</tbody>
</table>

*Total confirm cases PHI-Public Health Inspector

Results revealed that number of female cases are slightly higher than male cases (see Figure 1).

Figure 1. DF cases by gender

Results revealed that 6-15 age group has the highest number of cases in 2011 & 2012. (see Figure 2 & 3)

Figure 2. DF cases by age category 2011

Figure 3. DF cases by age category 2012

Results revealed that 6-15 age group has the highest number of cases in 2011 & 2012. (see Figure 2 & 3)

Figure 4. DF cases by hospital category
Results revealed that highest number of patients were admitted in government hospitals in both 2011 & 2012. (see Figure 4)

Table 2. Incidence of dengue fever according to gender and PHI areas

<table>
<thead>
<tr>
<th>Variable</th>
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<td>Female</td>
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<td>Saranankara</td>
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<tr>
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<td>22410</td>
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</tr>
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<td>7911</td>
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<tr>
<td></td>
<td>Kandawala</td>
<td>9076</td>
<td>7.16</td>
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</table>

*Incidence per 1000

Figure 5. Rainfall & Dengue trends during 2011

Figure 6. Rainfall & Dengue trends during 2012

Rainfall was correlated with DF confirmed cases, for 7 weeks preceding the registration period. There was significant correlation between rainfall and confirmed dengue cases, $r = 0.38$, $p < .01$, $R^2 = 0.144$, & rainfall accounts for 14.4% of the variability in DF. There were no significant relationship with humidity and temperature with dengue cases, $r = 0.01$ & $r = 0.11$.

We found more water collecting areas, shanty houses and poor sanitary conditions in Badowita PHI area when comparing observational data with other PHI areas.

V. DISCUSSION

The present study calculated the incidence of confirmed dengue cases according to gender and PHI areas. Females had higher incidence than males. This study provides further evidence of previous work indicating that females have higher incidence than males (Mendis 2011). Higher incidence has been reported in Badowita PHI area (14.50 per 1000) in both years. Lowest incidence for 2011 was reported from Mount Lavinia Central (2.26 per 1000) and for 2012 was from Dehiwala Central (1.48 per 1000). Incidence of Total MOH area, in 2011 was higher than 2012. Observational
data prove that Badowita PHI area has more breading places for Aedes aegypti and Aedes albopictus mosquitoes than other areas. Highest number of patients has admitted to government hospitals due to free service.

Notified date differs from the date the patient acquired the disease in some cases, due to the failure of hospitals to report the cases in time. Our data was obtained using the notification registers of MOH office. Most of the unconfirmed cases are due to inability to trace back to the patient, reporting of viral fever as DF, and patients were not living in the particular MOH area. There were only two stations to calculate rainfall data in Dehiwala MOH area. Therefore we took both readings to calculate the mean value. But this can differ from the rainfall in different PHI areas. And also humidity and temperature data was taken only from one station.

VI. CONCLUSIONS

This study suggested a positive correlation between rainfall patterns and dengue trends. Further, results indicated that dengue epidemic was started at the 7th week, after the highest rainfall. Although rainfall was positively correlated with dengue trends proceeding 7th weeks, it accounts for only 14.4% variation in rainfall. When considering $R^2$ value, this leaves 85.6% of the variability still be accounted for by other variables such as environmental factors, garbage disposal procedures, housing conditions, etc. Based on these findings we recommend that notification data of Dengue fever be analysed at MOH level to forecast outbreaks and intensify preventive measures.

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Ms J.D. Sachinthia Kumayasinghe is a 4th year medical student studying at South Asian Institute of Technology and Medicine. She received her higher education from Samudra Devi Balika Vidyalaya Nugegoda. She won merit award of inter school all island art competition in 2003 and distinction award in 2004. She participated inter school netball tournament in 2004 and inter house netball tournaments in 2003-2006.
Military Training Improves Lipid Profile

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Abstract - The effect of physical exercise during military training on plasma lipid levels has not been investigated in Sri Lankan military establishments. This study examined the effect of military training on lipid profiles of officer cadets in one of the training centres in Sri Lanka.

The study sample consisted of four groups; 1. Non-exercised control group (NE), 2. Military training for 6 months (E6M), 3. Military training for 18 months (E18M) and 4. Military training for 30 months (E30M). Each group consisted of 30 participants. Plasma HDL-cholesterol, triglycerides and total cholesterol (TC) were measured using enzymatic techniques and colorimetric determination. LDL-cholesterol level was calculated using the standard equation.

A significant reduction in mean TG was observed in all groups that had military training; E6M (p<0.005), E18M (p<0.05) and E30M (p<0.001) as compared to control. LDL-cholesterol levels were also significantly low in groups that had military training compared to the control group (p<0.005). The reduction in TC was significant in groups E18M (p<0.01) and E30M (p<0.005) and it was observed that prolonged exercise resulted in greater improvements in TC. HDL-cholesterol levels were significantly high in groups who underwent military training for more than 18 months (p<0.005 in E18M and p<0.01 in E30M).

Military training has significantly improved lipid profiles of young officer cadets.

Keywords— exercise, HDL-cholesterol, LDL-cholesterol, triglycerides, total cholesterol

I. INTRODUCTION

Life style changes due to industrialization and urbanization has led to many health issues. Non-communicable diseases are one such category. Excess weight during adult life significantly alters individual’s risk for cardiovascular disease (1,2) Hyperlipidaemia leading to atherosclerosis causes significant contribution to morbidity and mortality in many countries including Sri Lanka (3). Lack of physical exercises is a recognized contributory factor for hyperlipidaemia. Low plasma levels of high-density lipoprotein (HDL) cholesterol, and elevated low-density lipoprotein (LDL) cholesterol and triglyceride (TG) are important independent risk factors for atherosclerosis (4). Physical exercise has shown to improve lipid profiles by increasing insulin sensitivity and serum HDL cholesterol while decreasing serum LDL cholesterol and triglycerides (5).

Physical exercise is an essential component in military training. Military service requires strict adherence to body composition, fitness (6) and medical standards (7). Military personals are generally considered as healthy, physically-fit adults with a low risk for developing cardiometabolic disease. The officer cadets recruited to the General Sir John Kotelawela Defence University (KDU) undergo regular military training in addition to their academic work. Studies have not been carried out in Sri Lanka to investigate whether the physical exercise during military training improves plasma lipid levels. Hence, this study was carried out to investigate the effect of regular physical exercises on lipid profiles of officer cadets recruited to the KDU.
II. METHODS

A. Research design
The study sample consisted of gender matched four groups with 30 participants in each group. The ages ranged from 18 – 22 years. A non-military student group of the KDU with no regular exercise was taken as the control (NE). The other three groups had formal military training with regular exercise for varying durations; six months (E6M), eighteen months (E18M) and thirty months (E30M). Written informed consent was obtained from all the participants. The study protocol was approved by the Ethics Review Committee of the KDU.

B. Amount of physical exercise
The exercise groups participated in a supervised, progressive, strength training programme, with 45 minute to 2 hour sessions five days a week which included running, stretching and muscle strengthening exercises. In addition, a 45 minutes swim per week was also conducted.

C. Study protocol
5 mL of venous blood samples were drawn into heparinised tubes after 12 hours of overnight fast. The samples were centrifuged immediately at 1500×g for 15 min to separate plasma. Plasma samples were used to measure HDL-cholesterol, TG and total cholesterol (TC). HDL-cholesterol level was measured using HDL Cholesterol kit (Human, Germany). A TG kit (Biolab, France) was used to measure TG levels and cholesterol liquicolor kit (Human, Germany) was used to measure TC levels. All the reactions were carried out according to the manufacturer’s protocols and colorimetric determination was done at given wave lengths using a spectrophotometer. LDL-cholesterol was calculated by using the following standard equation; 

\[
LDL = TC - HDL - TG/5.0 \text{ (mg/dL)}
\]

D. Data collection
Information about the participants’ sleeping behavior, alcohol usage, smoking habits and medication usage were recorded in addition to collection of blood.

E. Statistical analysis
Statistical analyses were conducted using SPSS statistical software. ANOVA was used to evaluate the difference between mean values of exercise groups and controls. Significant levels were determined by Post Hoc comparison of NE with exercised groups.

III. RESULTS

Table 1: Mean height and weight of the participants

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean height (cM)</th>
<th>Mean weight (KG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE</td>
<td>168.8</td>
<td>67.3</td>
</tr>
<tr>
<td>E6M</td>
<td>169.7</td>
<td>59.1</td>
</tr>
<tr>
<td>E18M</td>
<td>172.1</td>
<td>58.7</td>
</tr>
<tr>
<td>E30M</td>
<td>168.8</td>
<td>63.0</td>
</tr>
</tbody>
</table>

A. Effect of military training on total cholesterol
Those who had 30 months regular exercises had 20% reduction in TC (181.3±7.6 vs 145±8 mg/dl; p<0.005; Fig 1). The reduction in TC was significant in all exercise groups E18M (p<0.01) and E30M (p<0.005) compared to the control group.

B. Effect of military training on HDL and LDL cholesterol
There was a strong trend towards a significant decrease in LDL cholesterol in the exercising groups (p<0.05) (Fig 2). Decrease in LDL seen in 30 months exercise group was 33.9% compared to the control group. HDL-cholesterol levels were significantly high in groups who underwent military training for more than 18 months (p<0.005 in E18M and p<0.01 in E30M) (Fig 3). In addition, E30M shows 38.9% increase in HDL compared to NE. Further, a significant decrease in the LDL to HDL cholesterol ratio was observed in the exercising groups.

C. Effect of exercises on triglycerides
The mean TG difference between control and exercise group was significant; E6M (p<0.005), E18M (p< 0.05) and E30M (p<0.001). The total reduction of TG at the end of the 30 months was 37.2% (Fig 4). However, there was an increase in the TG in E30M compared to the E18M.
IV. DISCUSSION

To the best of our knowledge, this is the first scientific research done in Sri Lanka to assess the effects of military training on lipid profiles. The present study clearly shows that the military training during the cadet life of officer cadets at the KDU induces a significant improvement in the lipid profiles. This finding is in agreement with reports of previous studies. A similar study was carried out in young men undergoing military service with a structured exercise training program (8). They reported a decrease in total cholesterol, LDL cholesterol, triglycerides, and decreased ratios of LDL/HDL cholesterol and total/HDL cholesterol after 12 months of resistance training.

In our study the improvements in lipid levels were observed after 18 months of regular exercise. This trend was particularly seen in total cholesterol, LDL and HDL cholesterol levels. These results support the efficacy of regular physical exercises in altering lipid profiles in young individuals.

Table 2: P values of one way ANOVA and means for different types of serum lipids of different study groups

<table>
<thead>
<tr>
<th>Biochemical parameter</th>
<th>NE</th>
<th>E6M</th>
<th>E18M</th>
<th>E30M</th>
<th>P One way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>181.3±7.6</td>
<td>178.4±5.3</td>
<td>167.5±7.1</td>
<td>145±8</td>
<td>0.01</td>
</tr>
<tr>
<td>HDL-Cholesterol (mg/dL)</td>
<td>40.1±6.5</td>
<td>38.8±1.3</td>
<td>44.3±1.7</td>
<td>55.7±5.9</td>
<td>0.000</td>
</tr>
<tr>
<td>LDL- Cholesterol (mg/dL)</td>
<td>129.3±9.9</td>
<td>128.1±5.7</td>
<td>120.2±7</td>
<td>85.4±9</td>
<td>0.000</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>95.7±5.4</td>
<td>55.1±3.1</td>
<td>40.4±3.3</td>
<td>60.1±3.3</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Values are mean± standard error.

NE, Non-exercised control; E6M, exercised for 6 months; E18M, exercised for 18 months; E30M, exercised for 30 months

P <0.001 for the comparison with the control group.
Diet is an important factor for lipid profile (9). Our study was conducted in a military setting and a dietary intervention was not included.

None of the participants had consumed alcohol or smoked during the past 24 hours prior to the experiment. All the participants had nearly 5 hours of sleep during the night before data collection.

The favorable alterations observed in this study in the lipid profile might be due to the comprehensive lifestyle modifications required during military training.

V. CONCLUSIONS

Military training improves the lipid profile; improvements are more significant when the duration of training reaches 30 months.

ACKNOWLEDGMENT

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Knowledge, Attitudes and Practices (KAP) among Blood Donors from Southern Sri Lanka

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Abstract—Behaviour of the blood donors plays a major role in maintaining continuous blood supply for transfusion services worldwide. At the same time it is a challenge to obtain safe blood from donors. A cross-sectional descriptive study was conducted by using a self administered, anonymous questionnaire to determine knowledge, attitudes and practices among blood donors coming to the blood bank in the largest tertiary care centre in the Southern Province, Sri Lanka which caters to a multi-ethnic community. This study included 339 randomly selected donors with age range from 18 to 57 with the mean age of 30 (SD 7.8). Majority of them were males (98.2%), unmarried (48.9%), Sinhala Buddhist (99.4%) and had post secondary education (92.9%). Average frequency of the blood donation was 4.6 (SD 3.4). 75.2% of them had previously donated blood (repeat donors) and 83.5% of repeat donors had their first donation before 30 years. 99 % of the donors knew at least one indication for blood transfusion. Majority of the donors stated that sexually transmitted diseases cannot be considered as a cause of transfusion transmitted disease and were unable to define “unprotected sex”. Mass media significantly contributed to gain knowledge on transfusion transmitted diseases. The main reason given for not to re-donate was “no benefit for self”. Regular donors tend to direct others for donation than occasional donors (P=0.001). Young, educated donors and low female contribution reported here is similar to other countries. Willingness for re-donation and low post donation complications are encouraging findings. Potentiality of donors for recruiting others for blood donation is a novel finding of this study.

Keywords—Blood donors, Sri Lanka, KAP

I. INTRODUCTION

Since the first recorded successful blood transfusion by Dr. Jean Baptiste Denis on 1667, transfusion medicine has made significant progress. Blood donation has no alternative. It depends on altruism of the abled humans (Fastag, 2013). To receive safe blood at the right time for the right disease is a Right of the patient. Obtaining and maintaining safe blood stocks for the ever increasing demands are challenges faced by transfusion services all over the world. Non remunerated, regular, voluntary donors are believed to be the best source for safe blood (WHO, 2014). But citizens from most of the countries do not enjoyed that privilege. Many transfusion services of the developing countries mainly rely on replacement donors or remunerated donors who carry high risk for a safe blood concept (WHO, 2014).

The motivation, recruitment, selection and retention are considered as the most important strategies to ensure safe and continuous supply of blood from voluntary, non remunerated blood donors (Tendulkar, 2014). It has been shown that donors’ behaviour plays a major role in various aspects of road to safe donation. Willingness for blood donation which is an expected behaviour of the blood donor is influenced by dynamic factors (Masser, 2008; McMahon, 2008). Transfusion transmitted diseases are a major risk associated with blood transfusion which is not completely prevented by existing procedures. Unprotected sexual intercourse is the predominant mode of transmission of main transfusion transmitted diseases. While motivating potential, appropriate donors, it is essential to promote self-deferral of the high risk donors in order to minimise transfusion transmitted diseases.
Studies have been conducted in various countries to assess the knowledge, attitudes and practices of the donors to evaluate their behaviour. (Gillespie, 2002; Lownik, 2012; Uma, 2013; Olaiya, 2004). Donor motivation campaigns are based on donors’ feedback and the factors prevailing in the country which can influence the willingness of the donors. At the same time, surveillance is needed to look at rejection of donors and factors affecting the willingness for re-donation.

The objective of this study was to assess the knowledge related to indications for blood transfusion and transfusion transmitted diseases, to evaluate attitudes of the donors for re-donation and to determine the donors’ practices to direct others for blood donation.

II. METHODS

A cross sectional descriptive study was conducted in the blood bank at the Teaching Hospital Karapitiya which is the largest tertiary care hospital in southern Sri Lanka. The ethical clearance was obtained from the ethical review committee of the Faculty of Medicine, University of Ruhuna, Sri Lanka. The consented blood donors eligible for blood donation were randomly selected. A pretested, self administered, anonymous questionnaire in Sinhala language was given during the pre and post donation period. The pre donation questions were designed to probe donors’ Sociodemographic details and their knowledge on transfusion transmitted diseases. As an additional factor knowledge related to thalassamia was also questioned. The post donation questionnaire mainly evaluated their willingness for re-donation and practices to direct others for donation. Statistical analysis was done by using SPSS software. The Mann – Whitney U test was used to examine the differences between two groups.

III. RESULTS

This study included 339 donors. Their age ranged from 18 to 57 years with the mean age of 30 years (SD 7.8 years). A majority of them were males (98.2%), unmarried (48.9%), Sinhalese Buddhist (99.4%) and had post secondary education (92.9%) (Table 1).

Age at first donation was 21.8 years among the married donors which was significantly lower than the 25.3 years for the same parameter in unmarried donors(p<0.02). Unmarried donors had 3.7 donations compared with married donor 5.2 donations which was also significantly lower (p<0.02). Donors who had education above G.C.E(OL) had their first donation at a significantly earlier age than others (22.7 Vs. 24.3 P= 0.05). 75.2% of them had previously donated blood (repeat donors) and 83.5% had their first donation before 30 years of age (Table 2). Mean age was significantly higher among repeat donors than new donors (31.4 Vs. 24.3 p< 0.01). Mean age at the first donation was 23.7 years (SD 5.6) and average frequency of blood donation was 4.6 (SD 3.4) among repeat donors. Age of the donor was positively correlated with the number of donations (r 0.147 p=0.19). The age at first donation of the repeat donors negatively correlated with education (r -0.157 p<0.05) and number of donations(r -0.199 p<0.01). Donors who had donated more than five occasions (regular donors) started donation significantly earlier than donors who had less than five donations (occasional donors) (21.9 Vs. 24.4 p<0.01).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N=339</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>333</td>
<td>98.2</td>
</tr>
<tr>
<td>Females</td>
<td>6</td>
<td>1.8</td>
</tr>
<tr>
<td>No response</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>162</td>
<td>47.8</td>
</tr>
<tr>
<td>Unmarried</td>
<td>165</td>
<td>48.9</td>
</tr>
<tr>
<td>No response</td>
<td>12</td>
<td>3.3</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>25</td>
<td>7.4</td>
</tr>
<tr>
<td>21-30</td>
<td>162</td>
<td>46.8</td>
</tr>
<tr>
<td>31-40</td>
<td>108</td>
<td>31.9</td>
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<td>41-50</td>
<td>39</td>
<td>11.5</td>
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<tr>
<td>&gt;51</td>
<td>5</td>
<td>1.5</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No education</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>Up to OL</td>
<td>15</td>
<td>4.3</td>
</tr>
<tr>
<td>Up to AL</td>
<td>175</td>
<td>50.4</td>
</tr>
<tr>
<td>Tertiary education</td>
<td>140</td>
<td>40.3</td>
</tr>
<tr>
<td>No response</td>
<td>8</td>
<td>4.7</td>
</tr>
<tr>
<td>Religion</td>
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<td></td>
</tr>
<tr>
<td>Buddhism</td>
<td>337</td>
<td>99.4</td>
</tr>
<tr>
<td>Hinduism</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>Islam</td>
<td>1</td>
<td>0.3</td>
</tr>
</tbody>
</table>
99% of the donors knew at least one indication for blood transfusion (Table 3). 76.2% of the new donors correctly mentioned HIV/AIDS as a transfusion transmitted disease which was significantly lower than the knowledge of repeat donors, on the same area \((p=0.011)\). 58.4% of the repeat donors and 63% of the new donors stated that sexually transmitted diseases (STD) cannot be considered as a cause of transfusion transmitted diseases (Table 4). Only nine donors (2.7%) were able to correctly define “unprotected sex”.

Table 2. Donor blood donation details

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N=339</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous donations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repeat donors</td>
<td>255</td>
<td>75.2</td>
</tr>
<tr>
<td>New donors</td>
<td>84</td>
<td>24.5</td>
</tr>
<tr>
<td>Number of donation by repeat donors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-5</td>
<td>185</td>
<td>72.5</td>
</tr>
<tr>
<td>6-10</td>
<td>49</td>
<td>19.2</td>
</tr>
<tr>
<td>&gt;11</td>
<td>21</td>
<td>8.2</td>
</tr>
<tr>
<td>Age at first donation of the repeat donors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>71</td>
<td>29.8</td>
</tr>
<tr>
<td>21-30</td>
<td>142</td>
<td>59.7</td>
</tr>
<tr>
<td>31-40</td>
<td>21</td>
<td>8.8</td>
</tr>
<tr>
<td>41-50</td>
<td>2</td>
<td>0.8</td>
</tr>
<tr>
<td>&gt;50</td>
<td>2</td>
<td>0.8</td>
</tr>
</tbody>
</table>

As additional information we have collected and evaluated donors’ knowledge related to thalassamia. 34.8% knew that thalassamia can be avoided by pre-marriage blood test. Statistically significant higher number of regular donors mentioned that thalassamia can be prevented by pre-marriage blood test than occasional donors \((p<0.01)\).

75.22% of the donors stated that mass media significantly contributed to gain knowledge on transfusion transmitted diseases. Television (40.4%) and newspaper (34%) were mentioned as main sources to gain knowledge related to transfusion transmitted diseases (Table-5). Only 3% of the donors mentioned that radio was contributed for their knowledge.

Four donors (1.17%) had minor complaints during post donation such as pain at the site of the puncture.

Table 3. Donors’ Knowledge on indication for transfusion

<table>
<thead>
<tr>
<th>Question</th>
<th>N=339</th>
<th>Repeat donors (n=255)</th>
<th>New donors (n=84)</th>
</tr>
</thead>
<tbody>
<tr>
<td>What are the instances that blood or blood components can be used as a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>treatment?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>complications after delivery of a baby</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>239</td>
<td>93.7%</td>
<td>75(89.2%)</td>
</tr>
<tr>
<td>No</td>
<td>11</td>
<td>4.6%</td>
<td>4(4.8%)</td>
</tr>
<tr>
<td>Don't know</td>
<td>5</td>
<td>1.7%</td>
<td>5(6%)</td>
</tr>
<tr>
<td>Victims of road traffic accidents</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>254</td>
<td>99.6%</td>
<td>82(97.6%)</td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td>0.4%</td>
<td>2(2.4%)</td>
</tr>
<tr>
<td>Don't know</td>
<td>0</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Cancer patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>201</td>
<td>78.8%</td>
<td>53(63.0%)</td>
</tr>
<tr>
<td>No</td>
<td>43</td>
<td>16.8%</td>
<td>20(23.8%)</td>
</tr>
<tr>
<td>Don't know</td>
<td>11</td>
<td>4.4%</td>
<td>11(13.2%)</td>
</tr>
<tr>
<td>Genetic blood disorders</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>84</td>
<td>32.9%</td>
<td>31(36.9%)</td>
</tr>
<tr>
<td>No</td>
<td>27</td>
<td>10.6%</td>
<td>17(20.2%)</td>
</tr>
<tr>
<td>Don't know</td>
<td>144</td>
<td>46.5%</td>
<td>35(42.9%)</td>
</tr>
<tr>
<td>Critical stages of Dengue disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>198</td>
<td>76.6%</td>
<td>53(63.0%)</td>
</tr>
<tr>
<td>No</td>
<td>37</td>
<td>14.5%</td>
<td>12(14.3%)</td>
</tr>
<tr>
<td>Don't know</td>
<td>20</td>
<td>8.9%</td>
<td>18(21.4%)</td>
</tr>
</tbody>
</table>

Table 4. Donors’ Knowledge on transfusion transmitted diseases

<table>
<thead>
<tr>
<th>Question</th>
<th>N=339</th>
<th>Repeat donors (n=255)</th>
<th>New donors (n=84)</th>
</tr>
</thead>
<tbody>
<tr>
<td>What are the diseases that can be spread by blood?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIDS/HIV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>225</td>
<td>88.2%</td>
<td>64(76.2%)</td>
</tr>
<tr>
<td>No</td>
<td>17</td>
<td>6.6%</td>
<td>10(11.9%)</td>
</tr>
</tbody>
</table>
Table 5. Source of Knowledge on transfusion transmitted diseases

<table>
<thead>
<tr>
<th>Question</th>
<th>N=339</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>How did you get knowledge on disease which can be transmitted by blood?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Radio</td>
<td>10</td>
<td>3%</td>
</tr>
<tr>
<td>2. Television</td>
<td>133</td>
<td>40.4%</td>
</tr>
<tr>
<td>3. Newspapers</td>
<td>112</td>
<td>34.0%</td>
</tr>
<tr>
<td>4. Brochures</td>
<td>3</td>
<td>0.9%</td>
</tr>
<tr>
<td>5. Other person</td>
<td>71</td>
<td>21.6%</td>
</tr>
</tbody>
</table>

64.6% of the donors have stated that they were willing for re-donation (Table 6). 40% of the repeat donors and the 19% of the new donors were not willing for re donation which was statistically significant (p = 0.001). The main reason given against re-donation was “no benefit for self” by both groups (Table 6). 63.6% of the occasional donors and the 50% of the regular donors stated their willingness for re-donation (P<0.05).

71.4 Of regular donors and 48.3% of occasional donors have directed others for blood donation (P=0.001) (Table 7). A majority of the regular donors believed that explaining benefits to society as the best strategy to direct others for blood donation. In contrast, occasional donors mentioned that accompanying donors to blood bank as the best strategy (Table 7).

Table 6. Donors’ attitudes towards re-donation

<table>
<thead>
<tr>
<th>Question</th>
<th>N=339</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Will you donate blood again?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repeat donors (n=255)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>152</td>
<td>59.6%</td>
</tr>
<tr>
<td>No</td>
<td>102</td>
<td>40%</td>
</tr>
<tr>
<td>No response</td>
<td>1</td>
<td>0.4%</td>
</tr>
<tr>
<td>New donors (n=84)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>67</td>
<td>79.8%</td>
</tr>
<tr>
<td>No</td>
<td>16</td>
<td>19%</td>
</tr>
<tr>
<td>No response</td>
<td>1</td>
<td>1.2%</td>
</tr>
</tbody>
</table>

Reasons for no re-donation

1. time wasting
   - Yes                          | 10    | 10%          |
   - No                           | 11    | 11.1%        |
   - No response                  | 1     | 1.2%         

2. not willing to reveal private details
   - Yes                          | 53    | 21.6%        |
   - No                           | 9     | 3.6%         |

3. no benefit for self
   - Yes                          | 3     | 3%           |
   - No                           | 1     | 6.3%         |

4. donation is difficult and painful
   - Yes                          | 22    | 22%          |
   - No                           | 3     | 3%           |

5. other reasons
   - Yes                          | 22    | 22%          |
   - No                           | 3     | 3%           |

Table 7. Repeat donors’ practices for directing others for blood donation

<table>
<thead>
<tr>
<th>Question and the response</th>
<th>N=339</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Did you ever direct a person for blood donation?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occasional donor (n=185)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>88</td>
<td>47.6%</td>
</tr>
<tr>
<td>No</td>
<td>93</td>
<td>52.4%</td>
</tr>
<tr>
<td>No response</td>
<td>4</td>
<td>2.1%</td>
</tr>
</tbody>
</table>

Regular donors (n=70)

| How did you direct a person for blood donation?                                             |       |              |
| 1. by explaining benefits for self                                                         | 1     | 0.6%         |
| 2. by forcing                                                                               | 0     | 0            |
| 3. by providing information                                                                 | 1     | 0.6%         |
| 4. by accompanying to blood bank                                                           | 47    | 26%          |
| 5. by explaining benefits for society                                                      | 74    | 40.9%        |

71.4 Of regular donors and 48.3% of occasional donors have directed others for blood donation (P=0.001) (Table 7). A majority of the regular donors believed that explaining benefits to society as the best strategy to direct others for blood donation. In contrast, occasional donors mentioned that accompanying donors to blood bank as the best strategy (Table 7).
IV. DISCUSSION

There is a paucity of information related to blood donors and blood donation in Sri Lanka. This study represent a sample of donors that consist of new donors, occasional donors and regular donors where each category possess unique features and dynamics related to blood donation. Low female contribution for blood donation is seen in our study which was also reported by other studies (Gillespie, 2002; Uma, 2013). Hollingsworth and Wildman has reported only 1% female donors at their donor population (Hollingsworth, 2004). Low donor turnover and temporary deferral conditions like anaemia, low weight was attributed for this low female contribution (Uma, 2013). Further studies are need to evaluate the low female contribution for blood donation in Sri Lankan settings. Majority of the donors are Sinhala Buddhist who represented nearly 94% of the population of the southern province of Sri Lanka (DCS 2014). This is similar to the findings of an all-island survey conducted in 1985 with the participation of 26,932 donors which had shown that 96% of the donors were from Sinhala Buddhist population (De Soysa, 1992). Donors from the young age group and from higher educational background seen in our study are similar to the findings of previous studies (Bharucha, 2005; Allain et al, 2008; Uma, 2013). Low mean age of the regular donors and the negative correlation of the age at first donation with number of donation signify that recruitment at young age will retain donors. We would like to suggest that motivation campaigns should target the young age group including school children to become donors when they become eligible for donation.

Blood donation is totally dependent on altruism of human beings. Boulware et al has identified that Lack of awareness of the need for donation is a potential reasons for ethnic and racial disparities in blood donation (Boulware et al, 2002). If the donor knows reasons for need of blood transfusion it may positively affect their attitudes for donation and becoming a regular donor. At the same time they would become a safe donor if they know about transfusion transmitted diseases which would cause harm for the recipients. This is a valuable point for recruiting and retaining young blood donors who tend to be healthy and motivated which will improve the long term safety and sufficiency of a country’s blood supply. The younger population is more likely to practice risk behaviours which may put them at higher risk of developing transfusion transmissible infections. These two aspects were probed simultaneously in this study. Majority of them knew indications for blood transfusion. 85.2% of the donors stated that AIDS/HIV can be transmitted through blood which is similar to Shah et al study done in India (Shah, 2007). Low level of knowledge was recorded with regard to sexually transmitted diseases (STD) as a cause of transfusion transmitted diseases in which 58.4% of the repeat donors and 63% of the new donors stated that STD cannot be considered as a cause of transfusion transmitted disease. Perception of STD being only transmitted by sexual contact would have partly contributed to these findings. Only 2.7% (n=9) of the donors were able to define “unprotected sex” in this study. These two findings suggest that donors’ knowledge in this population may not be up to standard to become a safe donor.

Post donation assessment has shown that majority of the donors are willing to donate blood again. The main reason given for not to re-donation was “no benefit for self” by donors which could be considered as negative effect. Previous studies have showed that nearly 75% of the donors reflected positive effects as an impact of the blood donation (Nilsson, 2003; Uma, 2013). Only four donors had minor complaints during post donation which much lower than values reported by previous studies (Uma, 2013; Nilsson, 2003). Counselling has been recommended as an effective tool to improve knowledge, attitudes and behaviour for blood donation (Kulkarni, 2014). Post donation counselling would reduce negative effects.

We have evaluated the practices of the donors to direct others for blood donation. Results have indicated that regular donors tend to direct other people for donation. This indicates that blood donors themself can be employed as a potential tool to recruit others for blood donation. This aspect has not been previously studied.

In conclusion, blood donors from southern Sri Lanka share common trends of young age, high educational background, and low female contribution with donors from other parts of the world. They have shown good knowledge related to indications for blood transfusion. Willingness for re-donation and low post donation complication are encouraging findings. Potentiality of donors for
recruiting others for blood donation is a novel finding of this study.

ACKNOWLEDGMENT
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REFERENCES


BIOGRAPHY OF AUTHORS

1 Author is a lecturer in Physiology, Department of Physiology, Faculty of Medicine, University of Ruhuna. He has worked as medical officer at blood bank, Teaching Hospital Karapitiya.

2 Author is a medical officer attached to Ministry of Health, Sri Lanka. Former medical officer attached to Anaesthesia/Intensive care unit, Teaching Hospital Karapitiya.

3 Author is a pre intern graduate from Faculty of Medicine, University of Ruhuna.
Provision of Mental Health Services through Community Partnership

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¹ nffernando@gmail.com, ² arosha24@gmail.com

Abstract—
Background - The World Health Organization signalled the urgent need for provision of community mental health services at its Global Forum for Community Mental Health. This project was innovated to utilise the already available state services to provide better mental health services through community partnership. Project was carried out in the catchment area for admissions to Unit 06 of National Institute of Mental Health (NIMH), Angoda from the Colombo District.

Aims - The aim of this project was to minimize treatment gap that is rampant in provision of mental health care services. Deinstitutionalization of the mentally ill is also intended.

Methods - A community mental health team is established under the Consultant Psychiatrist consisting of Medical Officer-Mental Health (MOMH), Community Psychiatric Nurse (CPN), Psychiatric Social Worker (PSW) and Occupational Therapist. The community is involved in planning, implementation, monitoring and evaluation of the programme. Community meetings were held in selected areas. Community Volunteers were recruited and a training done. The Volunteers went into the community and started active case detection. Upon new case detection the team consisting of above, visited to evaluate and plan treatment. The multitude of satellite clinics established are used to provide treatment and where necessary Depot injections are provided monthly at home. All patients are reviewed periodically by the MOOMH. CPNN and PSWW ensure tracking of all follow up patients with the aid of the volunteers. Volunteers themselves are assembled periodically for feedback and appreciation.

Results - Feedback from the community and clients indicate satisfaction. Clients are functioning in the community while on treatment and unnecessary admissions have been prevented. Community Volunteers are bringing in new clients to NIMH and other clinics for receiving of treatment.

Conclusions - Such a community partnership in provision of mental health care is greatly serving to reduce the treatment gap.

Keywords— Mental Health, Community Partnership, Community Psychiatry

I. INTRODUCTION

Traditionally, mental health services are provided through centralised health care services which are hospital based, disease oriented and provided by medical professionals on a one to one basis.

According to the World Health Organisation mental, neurological, and substance use disorders are common in all regions of the world, affecting every community and age group across all income countries. While 14% of the global burden of disease is attributed to these disorders, most of the people affected - 75% in many low-income countries - do not have access to the treatment they need (WHO). This indicates a significant treatment gap.

An innovative approach is needed to deal with this treatment gap. The Declaration of Alma Ata in 1978 states the following; “People have the right & duty to participate individually & collectively in the planning & implementation of their health care”. This indicates the need for community partnership in developing a health care plan.

Considering the need for a new initiative the Consultant Psychiatrist and multi-disciplinary mental health care team of Unit 06 of the National Institute of Mental Health Angoda developed a project with the hope of reducing the treatment gap in the catchment area for admissions to the unit from the Colombo District.
The Objectives of this project was,
- To decentralize a centralized mental health service
- To provide a community based service
- To ensure a patient friendly service
- To foster community partnership

The team was keen to use the existing resources of the ministry of Health for this purpose and not to spend any extra expenditure for this project.

II. METHOD

The initial pilot project was done in the Kaduwela Medical Officer of Health Area. Five villages under this area were selected initially. These five villages were recommended by the Medical Officer of Health, Kaduwela.

The five villages selected were,
- Dadigamuwa
- Thunandahena
- Singhapura – Hokandara
- Heenatikumbura – Thalangama North
- Weliwita

A team from the Unit 06 of the National Institute of Mental Health initially visited the five villages and they had consultative meetings with the villages. These were done with participation of many formal or informal village representatives. The needs of the villages were assessed through focus group discussions.

From each village Community Volunteers were recruited to undergo a capacity building training programme with regard to Mental Health. Five groups from the five villages were selected and each group underwent five days of training at the National Institute of Mental Health. Five Main topics which the World Health Organisation has endorsed as significant in Community Mental Health were discussed in detail. The five topics are,
- Dementia
- Alcohol and Substance Abuse
- Depression
- Psychotic disorders
- Somatoform disorders

Some other topics included side effects of drugs used in Psychiatry, Violence prevention, Use of Activity in mental illness treatment and horticulture therapy.

The training was mainly done by Consultant Psychiatrist of Unit 06. Teaching and Training methods included lectures, presentations, practical sessions and field visits. 58 volunteers completed the capacity building.

They are functioning as community level workers to provide basic mental health care in a supportive role.

Community Volunteers detect potential new cases in the community and refer them to the mental health team. They also follow up on patients who are already on treatment and try to prevent defaulting. They become supporters to the care givers of the mentally ill at times, providing the care givers with much needed respite.

To link with the community, the unit has developed a Community Mental Health Care Team which can work with the volunteers to provide community based care. The team is made up of Medical Officer- Mental Health (MOMH), Community Psychiatric Nurse (CPN), Psychiatric Social Worker (PSW), and Occupational Therapist (OT)

![Community Mental Health Care Team](image)

Figure 1: Community Mental Health Care Team

The Care team with the Community Volunteers organised Mental Health Camps in the selected areas and these camps were focussing on issues such as Untreated Mental illness, Problems due to substance abuse and prevention of domestic violence.

The Community Mental Health Care Team also does domiciliary visits. These visits are done for
- Tracing new patients for diagnosis and treatment initiation
- Tracing defaulters
- For routine follow up
- Giving IM Depot medication monthly
Currently over five domiciliary visit programmes are done for relevant catchment areas by the team. Each programme caters for around 30 clients. We also use this project to train all categories of health staff in mental health as well.

Further partnerships are also developed with other key stakeholders in community mental health care. Links are established with Public Health Midwives of the area, Divisional Hospitals of the area as well as the Police.

In addition, school mental health camps are conducted in collaboration with the Medical Officer of Health Office.

To ensure continuation and further development, the Volunteer groups are assembled at NIMH regularly to appreciate their service as well as to provide feedback.

The Volunteers also get an opportunity to communicate their observations and ideas regarding the programme.

This is an ongoing project with further development and newer initiatives being explored.

III. CONCLUSION

There is no funding involved as the programme is conducted using the existing health services in the area. This programme is serving immensely to reduce the treatment gap and undue admissions to the tertiary care wards and undue stay in hospital has been reduced.
POSTER PRESENTATIONS
An Anatomical Study of the Jugular Foramen and Its Variations in Dried Adult Human Skulls in Sri Lanka

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Abstract—The jugular foramen has a wide ethnic variation in the anatomy and is a well known area for pathological lesions such as glomus tumours, Schwannomas etc, where such lesions are approached by drilling the bone around the jugular foramen. The authors intended to describe the morphometry and anatomical variations of the jugular foramen and the possible dimensional distinction between the jugular foramen and the jugular fossa. A descriptive study of 24 skulls was done regardless of the gender, to describe the morphometry of jugular foramen and jugular fossa along with scaled photographs. Comparisons between the right & left jugular foramen/ jugular fossa and comparison of jugular foramen & jugular fossa on the same side were done using the student t-test. We found that the jugular foramen was present bilaterally in all the skulls studied. The mean ML diameter of the jugular foramen was larger in the right 16.02(±2.46) mm than in the left 15.46(±2.68) mm, which is compatible with previous studies; the mean AP diameters of jugular foramen were 8.28(±1.70) mm on left side and 6.84(±1.76) mm on right side. Therefore the jugular foramen is morphometrically different from jugular fossa at least from the AP diameter (t<0.05 bilaterally) and should be considered as two distinct anatomical structures rather than fossa as a section of foramen. The rest of the variations are possibly due to constitutional, racial, gender related or genetic factors and supports previous established data on Jugular foramen.

Keywords—Jugular foramen, Jugular fossa, septation

I. INTRODUCTION

The jugular foramen (J.Fr) is a fascinating skull opening or a bony channel consisting of a complex bony architecture which transmits many important neurovascular structures out of the base of the skull to the carotid space. The organization of the foramen is difficult to conceptualize because it varies in size and shape in different crania, from side to side in the same cranium, from its intracranial to extra cranial end in the same foramen, because of its complex irregular shape, its curved course, its formation by two bones, and the numerous nerves and venous channels that pass through it. (Hussain et al, 2010)

Since 1500 A.D many researchers including Versalius were intrigued by the variations in shape and for the jugular foramen. Versalius (1543) in his illustrations of base of skull has mentioned compartmentation of jugular foramen. Several studies including osteological, radiological and microdissections were performed to solve the mystery of compartmentation and variations in the anatomy of jugular foramen, which led to conflicting observations (Shifan et al, 2013). Among these variations, it is well known that the jugular foramen varies considerably in size and shape of the jugular vein (Weber & Mckenna, 1994). An enlarged dome of the foramen forming the accommodation for the jugular bulb is another anatomical variation (Hatiboglu & Anil, 1992). Other than that, various intrinsic abnormalities, multiple variations of adjacent vascular structures and pathological processes occurring in posterior cranial fossa such as intracranial menigiomas, parangangiomas, schwannomas, metastatic lesions and infiltrative inflammatory processes from surrounding structures such as the middle ear might be contributing to these variations in jugular bony canal (Idowa, 2004). Surgical resection is the treatment of choice in the majority of these cases. Advances in Microsurgical techniques have made possible the removal of advanced jugular foramen lesions which were once assumed to be inoperable (Hussein et al, 2010). As neurosurgeons become
bolder in approaching this region, the need for familiarity with the detailed anatomy of this region becomes greater.

Several studies which were done elsewhere have described variation in size of the foramen, variation of certain compartments such as anteromedial compartment and posterolateral compartment, bipartite and tripartite divisioning, relations and bridging bony tissue in the foramen (Weber & Mckenna, 1994). Only a few similar studies were undertaken in Sri Lanka. Therefore the aim of this study was to describe the anatomy of the jugular foramen in Sri Lankan skulls with its clinically important anatomical variations as it could provide important information about the anatomy of the jugular foramen for reliable surgical interventions in this area.

Figure 1. (A) Right side, dry anatomic specimen photograph delineating the jugular foramen (black arrows) and the jugular fossa (white arrows). Outside – Inside view from the anterior perspective. (B) Right side, dry anatomic specimen photograph delineating the jugular foramen (black arrows) and the jugular fossa (white arrows). Inside - Outside view from the posterior perspective. Note the difference between two. (CC- carotid canal; OC – occipital condyle)

II. MATERIALS & METHODS

The study was conducted in the Department of Anatomy, Faculty of Medicine – Ragama. 48 Jugular Foramina from 24 dried adult human skulls of Sri Lankan origin were studied regardless of the male & female sex. All skulls used did not have erosions in the measured area. A precisely calibrated, standard manual venire caliper (minimum reading of 0.02mm) and a divide were used for measurements. Scalded photographs were taken.

A. Inclusion criteria
Healthy Skulls with intact base ((i.e: Well dried skulls, Non eroded base of the skull from inner and outer surfaces, non fractured base due to any injury)

B. Exclusion criteria
The Skulls that have been eroded and deformed

C. Osteometric parameters
Following parameters were studied:
1) Side: Right or left
2) Measurements in jugular foramina (J.Fr) [fig 1 & 3]:
   2.1. Maximum anteroposterior diameter of the foramen
   2.2. Maximum mediolateral of the foramen
3) Measurements in jugular fossa (J.Fs) (fig 1 & 2):
   3.1. Maximum anteroposterior diameter of jugular fossa
   3.2. Maximum mediolateral diameter of jugular fossa
4) Height: If domed, height is measured from the summit of the dome to the inferior border of the fossa (fig 4)
5) Dome: The bony roof is related to the presence of superior jugular bulb (fig 5)
Figure 2. Outlining of J.Fs (dotted white line) and J.Fr (solid fill black line), measurements of jugular fossa: ML diameter (dashed double headed arrow) & AP diameter (Solid fill double headed arrow) OC - occipital condyle, CC - carotid canal

Figure 3. Outlining of J.Fr (dotted white line) and J.Fr (solid fill black line), measurements of jugular foramen: ML diameter (dashed double headed arrow) & AP diameter (Solid fill double headed arrow) OC - occipital condyle, CC - carotid canal

Figure 4. Coronal section through J. Fr. Height of the J.Fs from inferior border of the fossa ( ) to summit of the dome ( ) can be measured using the depth measuring stick of the venire calliper and a glass slide can be used to mark the inferior surface of the fossa. The bony roof is related to the presence of superior jugular bulb.

Figure 5. Presence of an obvious dome (black arrow)

6) Septations/bridging: Bony bridges dividing the foramen into compartment
   6.1. Presence or absence
   6.2. Bipartite/ tripartite form of the jugular foramen
   6.3. Complete or incomplete [fig 6 (A, B)]
7) Separate foramina for inferior petrosal sinus: A well defined opening with bony circumference present in the J.Fr. (fig 7)
8) Laterality: from fixed bony demarcations (mid point of anterior margin of the foramen magnum to most medial point of inlet to jugular fossa/ foramen). (fig 8)

III. RESULTS

The morphometric analysis of the current study revealed the following. The data were statistically analysed and tabulated.

The jugular foramen was present bilaterally in all the skulls studied. On examination it became apparent that most of the foramina were surmounted by an obvious bony roof and complete division of the jugular foramen by bony septations were not an uncommon finding.
Figure 6. (A) Complete septation in J.Fr (completion of the bony septum is shown by the black arrow); (B) Incomplete bony septation in J.Fr (incomplete bony septum is shown by the yellow arrow)

Figure 7. Showing separate foramen for IPS (inferior petrosal sinus)

Figure 8. The metric measurement of laterality. (J.Fr- Jugular foramen; OC- Occipital condyle; FM- Foramen magnum; L- Laterality)

A. Jugular foramen
1) Anteroposterior (AP) diameter: The mean AP diameters were 8.28(±1.70) mm on left side and 6.84(±1.76) mm on right side respectively. It was found that 67% of total foramina were having AP diameter between 5 and 10mm. A few of them, i.e. 16.5% of them had AP diameters less than 5mm; all of these narrow foramina were belonged to the left side, 16.5% were having AP diameter more than 10mm. However, the comparison between right and left AP diameter did not show any significant difference. A slit like Foramina was observed in one skull on the left side with AP diameter of 4.66mm and the ML diameter was 19.96mm.

2) Mediolateral (ML) diameter: The mean ML diameter was 16.02 (±2.46) mm in right side and 15.46 (±2.68) mm in left sides and no statistically significant difference was observed between the two sides. ML diameters ranged from 10.68mm to 21.18mm and most of the foramina, i.e. 77% were found to have a ML diameter between 13 to 19mm, 15% between 10 to 13mm and 8% were noted to have more than 19mm.

B. Jugular fossa
1) Anteroposterior (AP) diameter: The mean AP diameters were 6.85mm on left side and 8.3mm on right side respectively. The AP diameters ranged from 3.86mm to 16.12mm and 72.5% of total foramina were having AP diameter between 7 and 13mm. A few of them, i.e: 15% of them had AP diameters less than 7mm; all of these narrow foramina were belonged to the left side; the rest of the fossae were having AP diameter more than 13mm. Diameters on left and right did not show significant difference(t>0.05).

2) Mediolateral (ML) diameter: The observed mean values were 11.6mm on the left side and 12.6mm on the right and the diameters ranged 8mm on left and 8.3mm right.

C. Jugular foramen vs. jugular fossa
The AP diameter of the jugular fossa depicted significant difference from AP diameter of the jugular foramen (t<0.05 bilaterally). ML diameters of them did not depict such difference.

D. Height/depth of jugular fossa
These measurements ranged in a similar manner bilaterally and mean values had closer values, i.e. 11.6mm on left and 11.9mm on right, with no significant difference between them.

E. Dome of the jugular fossa
The prominent dome was noticed bilaterally in 7(29.16%) & unilaterally in 14(58.3%) skulls; out of unilateral prominent dome, 12(86%) were seen in right side.

F. Presence of septa
Usually the jugular foramen is partially divided to three compartments by two marked constrictions. Complete partition is not a common feature (Singla
et al, 2012). In the current study, all the observed 48 foramina had at least one septation, either complete or incomplete. Bilateral complete septation of jugular foramen were found in 3 skulls (12.5% of 24 skulls). Another 6 skulls (25 %of 24 skulls) had complete unilateral septation and 84% of them were on the left side. Out of all the jugular foramina, 37 (77%) foramina had either a complete or an incomplete single septa. 10 (21%) foramina were observed to have 2 septae regardless of completeness while 1 foramina showed 3 incomplete septae, dividing the foramina in to 4 incomplete compartments.

G. Separate foramina for inferior petrosal sinus (IPS)
A separate opening for IPS was observed in bilaterally in 3 skulls (12.5%) & unilaterally in 7(29.16%) skulls. Out of the latter category, 5(71%) separate IPS openings were observed on the left side.

H. Laterality
The observed mean laterality in current study were surprisingly, exactly similar up to the second decimal, i.e. 22.69mm on both sides and the values ranged 9.6mm on left and 11.7mm on right.

IV. DISCUSSION
Jugular foramen is located between the petrous portion of temporal bone and occipital bones, posterior to carotid canal and it connects the posterior cranial fossa and the jugular fossa. It lies in an oblique position, from the lateral aspect posteriorly toward the medial aspect anteriorly (Kenan & Ossama). From inner surface of the skull base to outwards, it courses anteriorly then laterally and finally inferiorly through the skull base. Anteriorly it is separated from the inferior carotid opening by a bony ridge, the carotico-jugular spine. The jugular foramen is lateral to the hypoglossal canal and the two are separated by an osseous bar and it serves as a passage for the glosopharyngeal, vagus and accessory cranial nerves, internal jugular vein, two dural sinuses and the meningeal branches of the occipital and ascending pharyngeal arteries (Shifan et al, 2013). According to Rhoton, the jugular foramen can be divided into 3 compartments (Rhoton et al, 1975; Linn et al, 2009);

1) A neural compartment (pars nervosa), containing the glossopharyngeal nerve;
2) A larger venous compartment (pars vascularis - sigmoid part), containing the sigmoid sinus and;
3) A smaller venous compartment (pars vascularis - petrosal part), containing the inferior petrosal sinus.

The sigmoid and the petrosal parts are separated by bony processes: the intrajugular processes, which originate from the opposing surfaces of the temporal and occipital bones, as well as by a dural septum, which connects these 2 bony structures. The smaller pars nervosa is relatively more consistent in size compared with the larger and more variable pars vascularis. Not all the cranial nerves pass through the pars nervosa as the name suggest. Only the glossopharyngeal nerve goes through the pars nervosa together with the inferior petrosal sinus. The vagus and accessory nerves travel with the jugular vein in the pars vascularis. Within the jugular foramen the glossopharyngeal nerve gives off the glomus bearing tympanic branch called the nerve of Jacobson (Hussain et al, 2010).

However, the jugular foramen is difficult to understand three dimensionally and difficult to access surgically; the difficulties in exposing this foramen is created by its deep location and the surrounding structures such as carotid artery anteriorly, the facial nerve laterally, hypoglossal nerve medially and vertebral artery inferiorly (Shifan et al, 2013). The size and shape of the jugular foramen is related to the size of the internal jugular vein and the presence or absence of a prominent superior jugular bulb. Standard text books suggests that the superior sagittal sinus being drained into the right transverse sinus, thus the right foramen is usually larger than the left, but there is a very wide variation in the anatomy of the intra cranial venous sinuses which accounts for variation in size and shape of jugular foramen (Woodhall, 1939). According to Padget (1957), the difference in size of the two internal jugular veins is already visible in the human embryo at the 23mm stage and probably results from differences in the pattern of development of the right and left brachiocephalic veins.

A study which looked in to these variations by Pereira et al (2010) mentions the mean AP diameter of the jugular foramen to be 9.21±1.95mm and 8.65±1.57mm and ML diameter to be 15.82±2.67mm and 15.86±2.64mm on right and left sides respectively in Southern Brazilian
population. They also mention that the AP diameter of J.Fr is significantly larger on right side and which may be related to prominent superior bulb of internal jugular vein. In an another study, Idowu (2004) reported the mean AP diameter to be 10.02mm and 9.57mm and mean ML diameter 13.9mm and 14.11mm on right and left side respectively. Current study gave slightly lesser mean diameters bilaterally on AP dimensions, than above studies. Also, in contrast to Pereira et al, AP diameters of left & right did not have a statistically significant difference. However, the ML diameters in current study were bigger than the measures by Idowu (2004) bilaterally and right ML diameter in Pereira et al; left side ML diameter of Pereira et al (2010) is almost equal with current study.

Considering the examined J.Fr, Sturrock (1988) states that the right J.Fr to be larger in 69% of skulls whereas Hatoboglu & Anil (1992) found that 61.6% were larger on right and 26% were larger on left. The current study observed 75% skulls with larger J.Fr on the right side and in the rest it was larger on left.

An unusual slit-like J.Fr on the left side with AP and ML dimensions of 2.47mm and 7.74mm respectively was reported by Rastogi and Budhiraja (2010). In current study authors observed a slit like foramina in one skull on the left side with AP diameter of 4.66mm and the ML diameter was 19.96mm which is well more than twice the size of the ML diameter than reported by Rastogi and Budhiraja (2010). According to Kawabe et al (2008), due to narrowing of foramen IX, X and XI cranial nerves may get involved resulting in Vernet’s syndrome. It might cause the neurovascular symptoms which can mimic the symptoms of jugular meningiomas or a glomus jugular tumor.

The jugular fossa is the other structure located in close relationship, hence confused due to the same reason, with the J.Fr. It is located at the inferior aspect (inferior surface) of the petrous part of the temporal bone as a deep bony depression, the size of which varies from a skull to skull. It communicates with the posterior cranial fossa via the jugular foramen. It lodges the jugular bulb which continues into the jugular vein inferiorly (fig 4). In the neurosurgical literature, and even in extensive anatomic studies, both the jugular foramen and the jugular fossa often are referred to by the term “jugular foramen.” This use of the term may be the result either of simple error or the user’s wish to provide a broader anatomic description of the area, and this confusion may be the underlying reason for current lack of agreement regarding the internal anatomic organization of “the jugular foramen” as well. Regardless of the reasons of this mix up, the jugular foramen and the jugular fossa are two distinct anatomic formations, although they are intimately related (Kennan & Ossama). No previous studies have been done to assess this fact comparing the dimensions of J.Fr and J.Fs. However, this fact could be strengthened statistically during the current study as the authors observed that the mophometric dimensions of J.Fr was significantly different than the dimensions of the J.Fs at least from their AP diameters(<0.05 bilaterally), hence should be considered as two distinct anatomical structures rather than J.Fs as a part of the J.Fr.

The depth of the J.Fs was measured as given in fig 4 and they were almost similar to the values observed by Singla et al, 2012. Although Singla et al (2012) has named this dimension as ‘the depth of the foramina’, the author prefers the term ‘the depth of jugular fossa’ for this measurement as it includes the dome which makes up the J.Fs. However, regardless of the given name, measurements in current study depicted no statistically significant difference between left and right sides. Most of the fossae had a depth between 5 – 15mm (82% of fossae with obvious domes). Authors also observed a deep tunnel like fossa which measured a depth of 19.62mm on right side, where as Singla et al (2012) mentions about a similar fossa of 24.23mm on right side.

The incidence of bilateral domed bony roof in current study was 29.16% which is much lesser than the percentages given by Pereira et al (2010) who reported bilateral roof in 68.5%; Sturrock (1988) reported the domed roof bilaterally in 53.9% and Singla et al (2012) observed 66%. But our values were closely related to the study by Patel and Singel (2007) who found this feature in only 29% of the skulls.

Vlajkovic et al (2010) observed bipartite form of J.Fr domination and had 24% of skulls out of 50 skulls with complete bridging. Meanwhile Sturrock (1988) and Hatiboglu and Anil (1992) observed right side complete bony septation in 5.6% and 3.2% skulls respectively. Pereira et al observed the bilateral
complete septation in 0.9% of cases. Singla et al (2012) observed complete bilateral and unilateral septum in 8% and 4% cases respectively. The current study gave different values than all of the above studies, observing complete bilateral and unilateral septations of 12.5% and 25% respectively.

V. CONCLUSION

The mean dimensions of the J.Fr were larger in the right side than in the left side and is compatible with previous regardless of local variations. J.Fr is morphometrically different from J.Fs at least from the AP diameter and should be considered as two distinct anatomical structures rather than J.Fs as a section of J.Fr. The rest of the variations are possibly due to constitutional, racial, sexual or genetic factors and supports previous established data of J.Fr. Knowledge of the observed variations is important for neurosurgeons, ENT surgeons, radiologists & Anthropologists.

VI. RECOMMENDATIONS

Considering this research as a pilot trial, there seems to be more room for further systematic studies in Sri Lanka to verify the spatial organization of the Jugular foramina and its internal anatomical variations precisely, using imaging techniques including high resolution CT/ MR angiography with the purpose of providing reliable anatomical information, to understand precise anatomical variations which will be important in neurosurgery and to add literature regarding the foramina in Sri Lankan population.

ACKNOWLEDGMENT

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Antioxidant Activity of Ingredients of Mathumeha Chooranam and Mathumeha Chooranam used in Mathumeham (Dibetes mellitus)

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Abstract- In the Siddha system of Medicine, various ‘chooranams’ are used to treat ‘Mathumeham’ (Diabetic mellitus). ‘Mathumeha chooranam’ is prepared from Terminalia chebula, Phyllanthus sembelica, Murrya keonigii, and Gymnema sylvestre. This ‘chooranam’ is widely used in North and Eastern Province of Sri Lanka Siddha Hospitals and Dispensaries. The Objective of this study was to determine the antioxidant activity of the ‘Mathumeha chooranam’ and its ingredients. The cold and hot water extracts (10mg in 10ml) of the dry powders of the skin of the seeds of Terminalia chebula, unripe fruits of Phyllanthus emblica, leaves of Murrya keonigii and Gymnema sylvestre, and ‘Mathumeha chooranam’ were tested for their Total Antioxidant Content (TAC) by measuring Ferric Reduction method.

Among the ‘mathumeha chooranam’ and its four ingredients, Terminalia chebula contained the highest TAC in cold as well as in hot extracts [10.13 (±3.1) and 12.83 (±2.4) µg/mg of dry weight respectively followed by ‘Mathumeha chooranam’ [4.6 (±1.16) and 5.6 (±0.91) µg/mg of dry weight respectively, Phylanthus emblica [4.38 (±1.72) and 6.3 (±2.05) µg/mg of dry weight respectively , Murryake onigii [0.506 (±0.372) and 0.696 (±0.336) µg/mg of dry weight respectively and Gymnema sylvestrae [0.359 (±0.262) and 0.759 (±0.665) µg/mg of dry weight respectively. The cold and hot aqueous extracts of the dried powder of the ingredients of the ‘mathumeha chooranam’ and its ingredients contains antioxidant activity. When compared with the cold extracts of ‘Mathumeha chooranam’ and its ingredients with hot extracts, hot extracts contained higher antioxidant activity than cold extracts.

Keywords: Antioxidant activity, ‘Mathumeha chooranam’, Ferric Reduction method, ‘Mathumeham’, Siddha Medicine

I. INTRODUCTION

Halli well and Gutteridge(1989) defined antioxidants as compounds that when present in low concentration in relation to the oxidant - prevent or delay the oxidation of the substrate. Free radicals are involved in many disorders like neurodegenerative diseases, cancer, aids and diabetes mellitus. Oxidative stress in cells and tissues results from the increased generation of reactive oxygen species and / or from decreases in antioxidant defense potential (Gumieniczek et al2002) Antioxidants works to maintain the oxidant at optimum level and to reduce free radical before disturbing living cells in our body

The symptoms of Diabetes mellitus can be correlated symptoms of Mathumeham. Diabetes mellitus is a metabolic disorder characterized by fasting hyperglycemia, and alteration in carbohydrate, fat and protein metabolism associated with absolute or relative deficiencies in insulin secretion and or insulin action (barar fsk 2000) Antioxidant actions are key to preventing or reversing Diabetes mellitus and its complications (Defronzo, R, 1999). Thus the aim of the present study was to evaluate the Antioxidant activity of the Mathumeha chooranam and its ingredients used in Mathumeham. Continuous use of synthetic anti diabetic drugs causes side effects and toxicity (Luo-2004, Alarcon et al 2004) Diabetes mellitus is known from ancient time onwards and numerous medicinal plants are used to control Diabetes mellitus in traditional medicine (Ajikumara et al 2006)

Mathumeha chooranam is widely used to treat Diabetes mellitus in Siddha hospitals and Dispensaries. This chooranam is prepared from the leaves of Gymnema sylvesrae, Skin of the seeds of Terminalia chebula,Fruit of Phyllanthus
embelica, and leaves of Murrya keonigii in 0.5:1:1:1 ratio respectively.

Gymnema sylvestre is an herb native to the tropical forests of southern and central India and Sri Lanka. It has been used to treat the Diabetes mellitus for nearly two millennia (Gurmar 2011). It belongs to the family of Asclepiadaceae. In Tamil it is called as Chakkaraikolli, In English small Indian epeccacuanha, and In Sinhala Bin nuga. Liubo 2009 reported that Gymnema sylvestre possesses Insulinotrophic activity of Human islets of langerhans. Shanmugasundram et al 1990 reported that Gymnema sylvestre has regeneration of the islets of langerhans in streptozotocin diabetic rats.

Phyllanthus embellica belongs to the family of Euphorbiaceae. In English it is called as Indian gooseberry, and in Sinhala called as nelli. Sandip et al (1999) stated that it has an antioxidant property. Another study with alloxan – induced rats given phyllanthus extract has shown significant decrease of the blood glucose, as well as triglyceridemic levels and an improvement of liver function (Qureshi SA et al 2009).

Murrya keonigii belongs to the family of Rutaceae. In English it is called as curry leaves, In Tamil karivepillai, and In Sinhala it is called as curryppincha. Tembhurne and Sarkarkar (2009) reported that it possesses antidiabetic activity. Iyer and Uma (2008); Chakarbarty et al 1997; and Tachibana et al 2003 demonstrated that the antioxidant carbozole alkaloids is presents in Murrya keonigii.

Terminila chebula is commonly called as black myrobalam, ink tree. It belongs to the family of compretacea. In English it is called as Chebulic myrobalan In Tamil Kadukkaai, In Sinhala Aralu. Ghandipuram Periyasamy et al (2006) reported that it possesses anti diabetic activity. Hazra B et al reported that it possesses the antioxidant and reactive oxygen species scavenging properties. Chia-Lin and Che-san Lin reported that it has an antioxidant activity.

V. MATERIALS AND METHODS

A. Plant material
Leaves of Gymnema sylvesre, leaves Murrya keonigii, Seeds of the Terminalia chebula and Fruit of Phyllanthus embelica were collected from Karaveddy and Meesalai of Jaffna peninsula.

B. Preparation of plant Extract
Leaves of Gymnema sylvesre, leaves Murrya keonigii, Seeds of the Terminalia chebula and Fruit of Phyllanthus embelica were cleaned, washed and dried under shade at room temperature for 10 days. The individual parts were powered and sieved with muslin cloth. Then stored in airtight container. Mathumeha chooranam was prepared from the above powders in 0.5:1:1:1 ratio respectively. Mathumeha chooranam and its ingredients (10mg) were used to prepare the cold and hot aqueous extract. 10mg of each powder was dissolved in 10ml distilled water and one part was kept in room temperature, other part was kept in water bath at 100°C for 5 minutes. Then these were centrifuged at 10,000 rpm for 10 minutes. Supernatant was taken from the centrifuged extract.

C. Determination of Antioxidant Activity
Total Antioxidant Activity was determined based on Ferric reduction method by using spectrophotometric method (Yildirm, et al, 2001) at monhly intervals for six month. For this analysis different volumes of 0.025ml, 0.050ml, 0.075ml, 0.100ml, 0.150ml, 0.200ml of each extracts were mixed with 0.5ml of 0.2mphosphate buffer (PH6.6) and 0.5ml of 1% Potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes, then rapidly cooled, mixed with 0.5ml of 10% Trichloroacetic acid and centrifuged at 6500 rpm for 10 min. An aliquot (1ml) of the supernatant were diluted with distilled water (1ml) and then 0.1% Ferric chloride (0.2ml) was added, vortexes and allowed to stand for 30 minutes. The absorbance were read spectrophotometrically measured at 700nm.

VI. RESULTS AND DISCUSSION

A. Ferric reduction method
TAC of the different cold and hot extracts are given in the table.

<table>
<thead>
<tr>
<th>Plant material</th>
<th>Total Antioxidant Content µg/mg</th>
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<tbody>
<tr>
<td></td>
<td>Hot extract</td>
</tr>
<tr>
<td>Terminalia chebula</td>
<td>12.83±2.4</td>
</tr>
<tr>
<td>Mathumehachooranam</td>
<td>5.6±0.91</td>
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</tbody>
</table>
Among the four ingredients of the mathumeha chooranam and mathumeha chooranam studied, Terminalia chebula showed highest total antioxidant content (TAC) in µg/mg of dry weight in cold as well as in hot extracts (10.13±3.1, 12.83±2.4)µg/mg of dry weight respectively followed by Mathumeha chooranam (4.6±1.16, 5.6±0.91)µg/mg of dry weight respectively, Phylanthus emblica (4.38±1.72, 6.3±2.05) µg/mg of dry weight respectively. Murrya keonigii (0.506±0.372, 0.696±0.336) µg/mg of dry weight respectively and Gymnema sylvestrae (0.359±0.262, 0.759±0.665) µg/mg of dry weight respectively.

The cold and hot aqueous extracts of the dried powder of the ingredients of the mathumeha chooranam And Mathuheha chooranam possess antioxidant activity. When compared with the cold extracts of mathumeha chooranam and its ingredients with hot extracts, hot extracts contained higher antioxidant activity than cold extracts. Among the four various ingredients, Terminalia chebula was found to possess significantly higher amount of antioxidant content than other ingredients.

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<table>
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<tr>
<th>Plant</th>
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<th>TAC Hot</th>
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<tr>
<td>Phylanthusemblica</td>
<td>6.3±2.05</td>
<td>4.38±1.72</td>
</tr>
<tr>
<td>Murryakeonigii</td>
<td>0.696±0.336</td>
<td>0.506±0.372</td>
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<tr>
<td>Gymnemasylvestrae</td>
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**Anti-Glycation and Glycation Reversing Potential of Salacia Reticulata L. (Kothala Himbutu) Root, Stem, Leaf and Twig Extracts**

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**Abstract**—Glycation is a series of complex reactions between reducing sugars and proteins. This reaction ultimately produces multitude of detrimental advanced glycation end products (AGEs). Formation and accumulation of AGEs have been implicated in the development and progression of several diabetic complications, neurological diseases and aging. Thus, glycation inhibitors and glycation reversing agents offer a potential strategy as therapeutics for diverse diseases. Salacia reticulata L. is a scientifically well documented traditional anti-diabetic plant. However, anti-glycation and glycation reversing potential of this plant has not been studied. Present study reports anti-glycation and glycation reversing potential of Salacia reticulata.

Freeze dried hot water extracts of Salacia reticulata root, stem, leaf and twigs were used in this study. Different concentrations of root, stem, leaf and twig extracts were subjected to anti-glycation and glycation reversing assays in vitro. Rutin was used as the positive control.

Root, stem, leaf and twig extracts of Salacia reticulata showed significant (P < 0.05) anti-glycation activity in a dose dependent manner. IC₅₀ values for anti-glycation activity of root, stem, leaf and twigs extracts were 13.06 ± 0.69, 27.29 ± 0.93, 144.53 ± 1.12 and 171.90 ± 0.88 µg/ml respectively.

Root extract showed significantly high (P < 0.05) anti-glycation activity compared to other extracts and rutin (IC₅₀: 21.88 ± 2.82 µg/ml). Glycation reversing potential of different parts of Salacia reticulata also showed significant (P < 0.05) and dose dependent relationship. EC₅₀ values of root, stem, twig and leaf extracts were 101.60 ± 11.57, 116.67 ± 0.64, 180.53 ± 7.41 and 264.40 ± 9.30 µg/ml respectively. Potency of different parts of Salacia reticulata for anti-glycation and glycation reversing activities were root > stem > leaf > twig and root = stem > twig > leaf respectively.

It is concluded that all parts of Salacia reticulata possess both anti-glycation and glycation reversing activities. Further, this is the first study to report anti-glycation and glycation reversing potential of this plant.

**Keywords**— Salacia reticulata, anti-glycation, glycation reversing

I. INTRODUCTION

Diabetes mellitus is a chronic metabolic disease increasing in epidemic proportions throughout the world (King et al., 1998). It affected about 171 million people worldwide in 2000 and the number is projected to increase to at least 366 million by 2030 (Wild et al., 2004).

This chronic disease is characterized by hyperglycaemia due to defects in insulin secretion or insulin resistance (Wild et al., 2004). Prolong hyperglycaemia results in the formation of advanced glycation end products in body tissues (Reddy & Beyaz, 2006; Wautier & Guillausseau, 2001). The complex AGEs formed can leads to protein cross linking and contribute to the development and progression of several diabetic complications such as peripheral neuropathy, cataracts, impaired wound healing, vascular damage, arterial wall stiffening, decreased myocardial compliances (Ahmed, 2005; Thomas et al., 2005; Aronson, 2003; Wautier & Guillausseau, 2001), neurological diseases and aging (Reddy & Beyaz, 2006). Thus, glycation inhibitors and glycation reversing agents offer a potential strategy as therapeutics for diverse disease conditions.

Salacia reticulata L. belongs to the genus Salacia and found in the submontane forests in Sri Lanka and India. In Sri Lankan and Indian traditional Ayurvedic medicine this plant is used in the treatment of variety of diseases. The roots and stems of Salacia reticulata have been extensively
used in the management of diabetes (Chandrasena, 1935). Traditional knowledge of anti-diabetic activity of roots and stems of this plant has been scientifically proven by many researches using in vitro, in vivo and clinical studies (Jayawardena et al., 2005; Kajimoto et al., 2000; Kumara et al., 2000; Shimoda et al., 1998; Yoshikawa et al., 1997; Yoshikawa et al., 1998; Serasinghe et al., 1990). However, extremely limited studies have been shown that bark and root extracts can reduce HbA1c levels in diabetes patients (Jayawardena et al., 2005; Kajimoto et al., 2000). Further, there are no studies on anti-diabetic activity of leaves and twigs of *Salacia reticulata*. We have previously shown that root, stem, leaf and twigs extracts of *Salacia reticulata* have anti-oxidant activity (Ranasinghe et al., 2007). As lot of oxidative reactions are known to participate in the process of AGEs formation (Reddy & Beyaz, 2006) different parts of *Salacia reticulata* may have anti-glycation and glycation reversing activities. Present study reports anti-glycation and glycation reversing potential of root, stem, leaf and twigs extracts *Salacia reticulata in vitro*.

II. METHODOLOGY

A. Materials
Leaves, stem, roots and twigs were collected from *Salacia reticulata* plantations of Eco-Tech Company Ltd at Nathandiya, Sri Lanka.

B. Chemicals and reagents
Bovine serum albumin (BSA), D-glucose and trichloroacetic acid (TCA) were purchased from Sigma-Aldrich, USA. All the other chemicals used for the preparation of buffers and solvents were of analytical grade.

C. Preparation of hot water extracts
Plant materials collected were air dried in an air-conditioned room (25 ± 2 °C) for 6 days. Then, air dried samples were ground to fine powder using a laboratory grinder. Two grams from powdered root, stem, leaves and twigs samples were extracted in 50 ml of hot water for 20 min. Extracts were then filtered, centrifuged at 6000 rpm for 10 min and freeze-dried (Christ-Alpha 1-4 Freeze dryer, Biotech International, Germany). Freeze-dried extracts were used in anti-glycation and glycation reversing assays.

D. Anti-glycation assay
The anti-glycation assay was performed according to the method of Matsuura et al. (2002) with some modifications. Freeze-dried extracts of *Salacia reticulata* root, stem, leaf and twigs (n=3 each) at 6 different concentrations (7.8, 15.6, 31.2, 62.5, 125.0 and 250.0 µg/ml) were used in the assay. Reaction mixtures containing 800 µg BSA, 400 mM glucose and different concentrations of *Salacia reticulata* extracts in a reaction volume of 1 ml in 50 mM phosphate buffer (pH 7.4) containing 0.02 % sodium azide (w/v) were incubated at 60 °C for 40 h. After cooling, aliquots of 600 µl were transferred to 1.5 ml eppendorf tubes and 60 µl of 100 % (w/v) TCA was added, stirred, centrifuged at 15,000 rpm at 4 °C for 4 min and supernatants were removed. The resulting AGEs-BSA precipitate was dissolved in 3 ml of phosphate buffer saline (pH 10) and fluorescence intensity was measured at an excitation wave length of 370 nm and emission wave length of 440 nm using a spectrofluorometer (Amino-Bowman*, Thermo Spectronic, USA). Rutin was used as the standard (positive control). Anti-glycation activity (inhibition %) of each *Salacia reticulata* extract and rutin was calculated using the following equation.

\[ \text{Inhibition} \% = \left[ \frac{(F_{c}-F_{a})-(F_{s}-F_{a})}{(F_{c}-F_{a})} \right] \times 100 \]

Where, \( F_{c} \) is the fluorescence of incubated BSA and glucose (control), \( F_{a} \) is the fluorescence of incubated BSA alone (blank), \( F_{s} \) is the fluorescence of the incubated BSA and glucose with *Salacia reticulata* extracts or the positive control (rutin) and \( F_{a} \) is the fluorescence of incubated BSA with the *Salacia reticulata* extracts or the positive control.

E. Glycation reversing assay
Reaction mixture containing 800 µg BSA and 400 mM glucose in 1 ml of 50 mM phosphate buffer (pH 7.4) containing 0.02 % sodium azide (w/v) was incubated at 60 °C for 40 h. After cooling, aliquots of 600 µl were transferred to 1.5 ml eppendorf tubes and 60 µl of 100 % (w/v) TCA was added, stirred, centrifuged at 15,000 rpm at 4 °C for 4 min and supernatants were removed. The resulting AGEs-BSA precipitates were dissolved in 50 mM phosphate buffer (pH 7.4) and added with *Salacia reticulata* extracts (15.6, 31.2, 62.5, 125.0 and 250.0 µg/ml; n=3 each) in a final reaction volume of 1 ml for incubation at 60 °C for 40 h. After cooling, 60 µl of 100 % (w/v) TCA was added, stirred and centrifuged at 15,000 rpm at 4 °C for 4 min. The resulting precipitates were dissolved in 3 ml of phosphate buffer saline (pH 10) and fluorescence
intensity was measured at an excitation wave length of 370 nm and emission wave length of 440 nm using a spectrofluorometer. Percentage glycation reversing was calculated using the following equation.

Glycation reversing (%) = \[ (F_c - F_b) - (F_s - F_{sb}) / (F_c - F_b) \] * 100

Where, \( F_c \) is the florescence of incubated BSA and glucose (control), \( F_b \) is the florescence of incubated BSA alone (blank), \( F_s \) is the florescence of the incubated BSA, glucose and Salacia reticulata extracts and \( F_{sb} \) is the florescence of incubated BSA with the Salacia reticulata extracts.

**F. Statistical analysis**

Data represented as mean ± SD (n=3). Data of each experiment were statistically analyzed using SAS version 6.12. One way analysis of variance (ANOVA) and the Duncan’s Multiple Range Test (DMRT) were used to determine the differences among treatment means. \( P < 0.05 \) was regarded as significant.

**III. RESULTS**

Anti-glycation and glycation reversing activities of Salacia reticulata root, stem, leaf and twigs extracts are given in Table 1 and Table 2 and Fig 1 and Fig 2.

**Fig 1.** Percentage of inhibition anti-glycation activity of Salacia reticulata root, stem, leaf and twigs extracts. \( IC_{50} \) values: root: 13.06 ± 0.69 \( \mu \)g/ml; stem: 27.29 ± 0.93 \( \mu \)g/ml; leaf: 144.53 ± 1.12 \( \mu \)g/ml; twig: 171.90 ± 0.88 \( \mu \)g/ml. \( IC_{50} \) values superscripted by different letters are significantly different at \( p < 0.05 \).

**Fig 2.** Percentage of inhibition glycation reversing activity of Salacia reticulata root, stem, leaf and twigs extracts. \( EC_{50} \) values: root: 101.60 ± 11.57 \( \mu \)g/ml; stem: 116.67 ± 0.64 \( \mu \)g/ml; twig: 180.53 ± 7.41 \( \mu \)g/ml; leaf: 264.40 ± 9.30 \( \mu \)g/ml. \( IC_{50} \) values superscripted by different letters are significantly different at \( p < 0.05 \).
Root, stem, leaf and twig extracts of *Salacia reticulata* showed significant (P < 0.05) anti-glycation activity in a dose dependent manner. IC$_{50}$ values for anti-glycation activity of root, stem, leaf and twig extracts were 13.06 ± 0.69, 27.29 ± 0.93, 144.53 ± 1.12 and 171.90 ± 0.88 µg/ml respectively. Root extract showed significantly high (P < 0.05) anti-glycation activity compared to other extracts and rutin (IC$_{50}$: 21.88 ± 2.82 µg/ml). Potency of different parts of *Salacia reticulata* for anti-glycation activity was root > stem > leaf > twigs.

Glycation reversing potential of different parts of *Salacia reticulata* also showed significant (P<0.05) and dose dependent relationship. EC$_{50}$ values of root, stem, twigs and leaf extracts were 101.60 ± 11.57, 116.67 ± 0.64, 180.53 ± 7.41 and 264.40 ± 9.30 µg/ml respectively. Potency of different parts of *Salacia reticulata* for glycation reversing activity was root = stem > twig > leaf.

<table>
<thead>
<tr>
<th>Salacia reticulata extract</th>
<th>Concentration (µg/ml)</th>
<th>IC$_{50}$ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7.8</td>
<td>15.6</td>
</tr>
<tr>
<td>Root</td>
<td>32.09 ± 3.47</td>
<td>59.55 ± 5.42</td>
</tr>
<tr>
<td>Stem</td>
<td>11.25 ± 4.46</td>
<td>22.85 ± 4.69</td>
</tr>
<tr>
<td>Leaf</td>
<td>12.44 ± 1.24</td>
<td>13.95 ± 2.77</td>
</tr>
<tr>
<td>Twigs</td>
<td>0.57 ± 0.63</td>
<td>0.49 ± 0.82</td>
</tr>
</tbody>
</table>

Data represented as mean ± SD. IC$_{50}$ values superscripted by different letters are significantly different at p < 0.05.

<table>
<thead>
<tr>
<th>Salacia reticulata extract</th>
<th>Concentration (µg/ml)</th>
<th>EC$_{50}$ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15.6</td>
<td>31.2</td>
</tr>
<tr>
<td>Root</td>
<td>8.50 ± 1.50</td>
<td>21.73 ± 1.83</td>
</tr>
<tr>
<td>Stem</td>
<td>5.71 ± 1.81</td>
<td>18.15 ± 2.84</td>
</tr>
<tr>
<td>Twigs</td>
<td>2.56 ± 1.74</td>
<td>8.05 ± 2.74</td>
</tr>
<tr>
<td>Leaf</td>
<td>8.50 ± 1.50</td>
<td>15.23 ± 3.41</td>
</tr>
</tbody>
</table>

Data represented as mean ± SD. EC$_{50}$ values superscripted by different letters are significantly different at p < 0.05.
IV. DISCUSSION

Protein glycation (Maillard reaction) is a series of complex reactions between carbonyl groups of reducing-sugars with amino groups of proteins. Amino groups of proteins react initially with reducing sugars to form Schiff bases followed by their Amadori rearrangement products. These Amadori products undergo a rearrangement reaction giving a multitude of end products that are known as AGEs (Reddy & Beyaz, 2006). Some of the AGEs are intensely coloured compounds and have typical fluorescence characteristics (Reddy & Beyaz, 2006). Therefore, in this study anti-glycation and glycation reversing activities were measured in terms of inhibition of Maillard fluorescence formation.

Findings of this study clearly showed that all parts of *Salacia reticulata* possess anti-glycation activity. Interestingly, root extract had significantly high anti-glycation activity compared to the positive control, rutin. Therefore, especially root extracts can be used in the management of AGEs associated chronic diseases. This plant is a well known anti-diabetic plant in Sri Lankan traditional knowledge and Indian system of Auyrveda (Chandrasena, 1935). Anti-diabetic activity of this plant has been explained through variety of mechanisms. However, anti-diabetic activity with respect to anti-glycation activity has been very poorly documented. Therefore, findings of this study showed a novel way of explaining the anti-diabetic mechanisms of this plant.

Glycation reversing is the reversing of already formed AGE cross links. It is an approach to attenuate AGE related complications. Such protein crosslink breakers might be useful as therapeutics for regulation of complications resulting from diabetes, neurological diseases and aging (Reddy & Beyaz, 2006). However, only few AGE crosslink breakers known to date and there are reports of their limited efficacy in *in vivo* studies (Reddy & Beyaz, 2006). Therefore, it is vital to explore compounds with AGEs reversing ability to manage AGE related complications. Findings of this study clearly showed that all parts of *Salacia reticulata* possess glycation reversing activity. These novel anti-diabetic properties further add values for this traditional medicinal plant as an anti-diabetic plant with multiple mechanisms. This is the first report of simultaneous presence of anti-glycation and glycation reversing activities of this plant.

Interesting and valuable findings of this study are that the presence of anti-glycation and glycation reversing activities in leaves and twigs. Therefore, leaves and twigs which can be repeatedly harvested in short cycles unlike root and stem can be used as a good natural source with anti-glycation and glycation reversing activities.

Different AGE inhibitors suppress AGE formation at different stages of glycation. Aspirin inhibits protein glycation at the early stage of glycation process by acetylating free amino groups of protein. Therefore, it causes to block the attachment of reducing sugars (Malik & Meek, 1994). The inhibitory activities of vitamin B1 and B6 derivatives such as pyridoxamine and thiamine pyrophosphate (Reddy & Beyaz, 2006; Booth *et al.*, 1996) have mainly been attributed to their abilities to scavenge reactive carbonyl compounds. We have previously shown that all parts of *Salacia reticulata* possess anti-oxidant activity (Ranasinghe *et al.*, 2007). Therefore, anti-glycation and glycation reversing activities may be due to the presence of anti-oxidative compounds. However, it is difficult to decide exactly at which stage of glycation process or in what way the intervention by *Salacia reticulata* extracts is exerted to reduce the glycation reaction. Further experiments are necessary to identify active compound/s, *in vivo* efficacy and mode of actions.

V. CONCLUSIONS

It is concluded that all parts of *Salacia reticulata* possess both anti-glycation and glycation reversing activities. Roots and stems were the most biologically active parts of the plant. Further, leaves and twigs, which can be repeatedly harvested in short cycles unlike roots and stems can be used as a good natural source with anti-glycation and glycation reversing activities. This is the first study to report anti-glycation and glycation reversing potential of this plant.

ACKNOWLEDGMENT

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Antihistamine Effect of Bee Honey in Wistar Rats

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Abstract - According to the Ayurveda classic Ashtanga Hridaya, written around 500 AD, honey can be used to treat many diseases. Eight types of honey are mentioned in Ayurvedic authentic texts. Out of these eight types of honey, the variety produced by honey bees is the most commonly referred to and is the type of honey consumed by humans. Honey produced by other bees and insects has distinctly different properties. Anti-inflammatory, anti-pyretic and anti-noceceptive effects of bee honey were established in Wistar rats in our recent experimental studies. In the present study, our aim was to evaluate the antihistamine potential of bee honey in Wistar rats, in order to elucidate one mechanism of anti-inflammatory action. The antihistamine effect of bee honey was compared with distilled water and chlorpheniramine controls in 3 groups of Wistar rats (n=6 in each). One hour after drug and bee honey administration, these rats were subcutaneously injected with 50 μl of 200 μg/ml of histamine dihydrochloride into the skin where the fur had been shaved, and 2 minutes later the area of the wheal formed was measured and percentage reductions in wheal area were calculated. The results of the study showed the bee honey induced an inhibition of wheal formation in the test group (31.0%) which was not statistically significant (p>0.05). The chlorpheniramine treated group showed 40.0% reduction in wheal formation when compared to the negative control group and it was statistically significant (p < 0.05). This study reveals that antihistamine effect is not a mechanism of anti-inflammatory activity of bee honey.

Keywords: Antihistamine, wheal formation, Wistar rats

I INTRODUCTION

The usage of honey as a medicine is referred in many ancient written records [1, 2]. Honey was prescribed by the physicians of many cultures for a wide variety of ailments [3]. It is mentioned in Rigveda [4] and is extensively used in Ayurveda [1,2] specially as a carrier of drugs.

Eight types of honeys are mentioned in Ayurveda [5]. They are Maksika (Bees honey), Bramara (honey produced by Bumble), Agra (honey produced by Wasp), Pouthika (honey produced by tiny insect call Kannei), Ouddhalaka (honey collected in anthill), Kshaudra (honey produced by species of tiny bees), Dala (honey collected in flower petals) and Chatra (honey collected by a certain kind of bees whose hive looks like an umbrella).

Out of these eight honeys, the variety produced by honey bees is the most commonly referred type of honey. Bees honey is recommended as an Anupana (vehicle) in paediatrics age groups in Ayurveda [6,7].

According to the Ayurvedic authentic texts, bee honey is widely used in the treatment of ophthalmic disorders, jaundice, piles, tuberculosis, asthmatic conditions and, respiratory disorders. Old bees honey helps to reduce over weight.

Anti-inflammatory effect of bee honey was established in Wistar rats in our recent experimental studies.

Literature survey revealed that bee honey is extremely useful as a carrier in Ayurvedic medicine but no scientific evidence is available for its antihistamine potential. Thus, in this present study antihistamine activity of bee honey was evaluated.
II MATERIALS AND METHODS

A. Bee honey;
Fresh bee honey was collected from Millaniya division in Kalutara district.

B. Animals
Healthy adult male Wistar rats (200–250 g) were used in the study. The animals were kept in plastic cages (two per cage) under standardized animal house conditions (temperature, 28–31°C; photoperiod, approximately 12 h natural light “per day”; relative humidity, 50–55%) with continuous access to pelleted feed and tap water.

All experiments in rats were carried out in accordance with the recommendation of the guidelines for care and use of laboratory animals and the project proposal was approved (No.591/11) by the Ethics Review Committee of the Faculty of Medical Sciences of the University of Sri Jayewardenepura, Sri Lanka.

C Antihistamine effect [(Spector W.G. 1956), (Rathnasooriya WD et al., 2005)]

Eighteen Wistar rats were randomly assigned into three groups (n = 6 in each). The left posterior lateral side of their skin was cleanly shaved. Group I served as negative control (received Distilled water), Group II received the positive control standard drug (Chlorpheniramine, 0.67 mg/kg) and Group III received bee honey. After 1 hour of drug administration, these rats were subcutaneously injected with 50 µl of 200 µg/ml histamine dihydrochloride (Fluka, Buchs, Switzerland) into the skin where the fur had been shaved, and 2 minutes later the area of the wheal formed was measured and % reductions were calculated.

III RESULTS AND DISCUSSION

A. Evaluation of antihistamine activity
The results of the study showed the bee honey induced an inhibition of wheal formation in the test group (31.0%) which was not statistically significant (p > 0.05). The chlorpheniramine treated group showed 40.0% reduction in wheal formation when compared to the negative control group and it was statistically significant (p < 0.05).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Percentage reductions in wheal formed %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group II – Chlorpheniramine</td>
<td>40.0</td>
</tr>
<tr>
<td>Group III – Bee honey</td>
<td>31.0</td>
</tr>
</tbody>
</table>

Table 1: Percentage reductions in wheal formed in Chlorpheniramine, and Bee honey groups in comparison to Negative Control.

IV CONCLUSION
This study reveals that antihistamine effect is not a mechanism of anti-inflammatory activity of bee honey.

ACKNOWLEDGMENT
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Prevalence of *Escherichia coli* and *Salmonella* on Different Cuts of Retail Chicken Meat in Badulla District

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Abstract—The present study was undertaken to determine the *Escherichia coli* and *Salmonella* contamination on different cuts of retail chicken meat from Badulla district. Twenty retail shops were randomly selected from seven secretary divisions in Badulla district. Two whole chicken samples were collected from each retail shop. Collected samples were screened for bacteria by selective culture procedure and presumptively positive colonies were bio-chemically confirmed. Prevalence of *Salmonella* in thigh, breast, back and wing cuts were 28.92 %, 20.48 %, 19.28 % and 13.25 % respectively. Prevalence of *Salmonella* in whole chicken sample was 18.07 %. No significance association was observed for the prevalence of *Salmonella* with different chicken meat cuts (P >0.05). Prevalence of *Escherichia coli* in thigh, breast, back and wing cuts were 20.99 %, 25.93 %, 24.69 % and 11.11 % respectively. Prevalence of *Escherichia coli* in whole chicken sample was 17.28 %. There was a significance association between chicken part and the prevalence of *Escherichia coli* in retail chicken meat in Badulla District. The highest occurrence of *Salmonella* was reported in Badulla division (19.28 %). An incidence of *Escherichia coli* (24.05 %) was significantly high in Bandarawela division. Prevalence of *Salmonella* in retail chicken meat in Badulla district is in high level and contamination of *E. coli* indicate the improper handling and storage of raw chicken meat and poor level of hygienic condition in retail outlets.

Keywords—Prevalence, Chicken, retail market

I. INTRODUCTION

Food safety is a global challenge for most developing countries. Microbes are developing resistant and able to survive many food productions and processing stages. Moreover they act as a potential threat to human health by occurring food borne illnesses (Akahtar *et al.*, 2012). Reported food borne diseases are mainly caused by bacteria such as *E.coli*, *Salmonella* and *S.aureus*. Animal originated foods are identified as an important source for transmission of these pathogens to human (Thilakarathna *et al.*, 2012).

Poultry industry in Sri Lanka has developed into a commercial industry over the past three decades from the back-yard system mainly due to active participation of the private sector. About 70% of the contribution to livestock sub-sector in Sri Lanka comes from chicken meat and the industry is capable of producing all local requirements of chicken meat (Ministry of Livestock & Rural Community Development, 2012). Chicken meat is relatively cheap compared to other animal products. Due to this, these products become the most consumed animal protein sources in the average Sri Lankan diets. Chicken meat is available throughout the country, in supermarket chains in the main cities and also small retail shops in rural areas. Current per capita availability of chicken meat estimated to be 4.8 Kg. Chicken meat is marketed from 15 large and medium scale broiler processors. Still four broiler processors and five further processing companies have obtained certification under internationally accepted HACCP system (Ministry of Livestock & Rural Community Development, 2012).

Small scale farmers send poultry carcasses without processing. People who handle the carcasses have not enough knowledge about proper hygienic practices during slaughtering and carcasses handling at retail market. Above factors are affect for the contamination of chicken carcasses at different stages. Incidence of diseases by contaminated food is in considerable level in Badulla district. According to the statistical data, there are 20% reported cases on food poisoning cases in Badulla district. Also the symptoms and signs involving the digestive system and abdomen are coming under top ten causes of hospitalization (Annual report of Provincial General Hospital -
Badulla, 2010). Therefore inspection of food borne pathogens in different cuts of chicken meat in small scale retail shops in Badulla District are important to indicate the hygienic quality and the safety of such meat for human consumption.

II. MATERIALS AND METHODS

A. Sample Collection
Samples were collected during April to August 2013. Twenty (20) retail shops were randomly selected from seven (7- Badulla, Ella, Bandarawela, Haliela, Haldummulla, Passara and Welimada) Divisional Secretary’s Divisions in Badulla District to collect the samples. Two (2) whole chicken samples were collected from each retail shop and packed separately and immediately transferred to the laboratory under refrigerated condition.

B. Preparation of Samples
Collected meat samples under refrigeration were thawed under aseptic condition at the laboratory. Then the whole chicken was separated in to parts such as breast, back, thigh, wings and whole. 25 g of meat from each separated parts were taken to analyse the microbiological quality.

C. Isolation of E. coli and Salmonella
Each meat sample was pre enriched with 225 ml of buffered peptone water and placed in incubator at 37 °C for 24 hours. Loops full of pre enriched samples were streaked on Eosin Methylene Blue agar and Brilliant Green agar to isolate E. coli and Salmonella respectively. Inoculated plates were incubated at 37 °C for 24 hours. Presumptive colonies on each agar plate, sub cultured on nutrient agar plates and incubated at 37 °C for another 24 hours.

D. Confirmation of E. coli and Salmonella
Presumptively positive colonies of E. coli, Salmonella on nutrient agar plates were biochemically confirm with Simmons Citrate agar. Presumptive colonies of E. coli and Salmonella on nutrient agar media were slightly inoculated on simmons citrate agar slant and incubated at aerobic condition at 37 °C for 24 hours. Salmonella are positive for citrate test and it will change the colour of agar media. But E. coli are negative and there is no any colour change of the agar media.

E. Data Analysis
Collected data were analyzed using Minitab 14 statistical software and the level of significance was set at 0.05.

III. RESULTS AND DISCUSSION
Out of 200 analyzed samples, prevalence of E. coli and Salmonella in different cuts of retail chicken meat in Badulla district is presented in Table 1.

Table 1. Contamination rate of bacteria on different chicken cuts

<table>
<thead>
<tr>
<th>part of the chicken</th>
<th>E. coli no of positive samples</th>
<th>E. coli %</th>
<th>Salmonella no of positive samples</th>
<th>Salmonella %</th>
</tr>
</thead>
<tbody>
<tr>
<td>breast</td>
<td>21</td>
<td>25.93</td>
<td>17</td>
<td>20.48</td>
</tr>
<tr>
<td>back</td>
<td>20</td>
<td>24.69</td>
<td>16</td>
<td>19.28</td>
</tr>
<tr>
<td>thigh</td>
<td>17</td>
<td>20.99</td>
<td>24</td>
<td>28.92</td>
</tr>
<tr>
<td>wings</td>
<td>9</td>
<td>11.11</td>
<td>11</td>
<td>13.25</td>
</tr>
<tr>
<td>whole</td>
<td>14</td>
<td>17.28</td>
<td>15</td>
<td>18.07</td>
</tr>
</tbody>
</table>

Total number of chicken meat samples that was positive for the Salmonella was 83 and it was 41.50 %. From that 28.92 % were thigh samples. It was the highest. Prevalence of Salmonella in breast part was 20.48 %. Prevalence of Salmonella in back and whole parts were 19.28 % and 18.07 % respectively. 13.25 % was belongs to prevalence of Salmonella in wing samples. It was the lowest. No significance association was observed for the prevalence of Salmonella with different chicken meat cuts.

Contamination rate of E. coli was 40.50 %. From that highest prevalence of E. coli belongs to breast part. It was 25.93 %. Prevalence of E. coli in back, thigh and whole parts were 24.69 %, 20.99 % and 17.28 % respectively. From the total positive samples 11.11 % belongs to wing samples. It was the lowest. There is a significance association between chicken part and the prevalence of Escherichia coli in retail chicken meat in Badulla District.

The highest occurrence of Salmonella was reported in Badulla division and it was 19.28% from positive
samples (16/83). An incidence of E. coli (24.05%) was significantly high in Bandarawela division.

According to the above results prevalence of Salmonella in retail chicken meat in Badulla district is higher than the prevalence of E. coli in retail chicken meat in Badulla district. There are several reasons affecting for the higher prevalence of Salmonella in retail chicken meat in Badulla district. In Badulla district there are 290 broiler farms and from that 249 broiler farms are coming under small scale farms (Department of census and statistic, 2012). During slaughtering of birds in small scale slaughter house, birds are killed and then scalded in hot water. The carcasses are then plucked and eviscerated, mostly by hand. Before and after evisceration, carcasses are often washed it is contribute to the prevalence of bacteria on and among carcasses. Also at the slaughtering cross contamination can be happen between positive and negative flocks, residual contamination from unclean equipments and through carrier workers and pests. The workers haven’t knowledge about proper hygienic practices during carcasses handling it is also affect the contamination of carcasses.

There have been a number of studies on meat hygiene in different countries. According to Adesiji et al., 2011, contamination rate of E. coli and Salmonella spp were 26% and 2% respectively in Nigeria. The findings by Lidija et al., 2006 with regard to microbiological quality and contamination of chicken meat, Enterobacteria (34.84%) and Salmonella spp. (10.60%) contamination rate in considerable level. Above results express the more contamination of E. coli and Salmonella on retail chicken meat in some countries. They used modern equipments for slaughtering process but hygienic practices are not well established.

Although, there is no significance association between prevalence of Salmonella in different chicken cuts, it is high in thigh (28.92 %) cut. Thigh part is proximity to the point of evisceration and due to improper evisceration it is highly prone to contamination of gut content. Similar results were found by Wilfred et al., (2010). The prevalence of E. coli indicates poor hygienic condition and working practices of the meat handlers during carcass handling and lack of sterilization of utensils and working surfaces of the retail market (Adesiji et al., 2011).

Different levels of contamination were observed in different divisions of Badulla district. Contamination rate of raw meat at retail shops differ with the climatic condition, storage temperature, refrigeration condition during transportation and the way of handling of raw meat (Van et al., 2007). Undeveloped slaughter facilities in country, large number of small scale retail businesses, lack of rules and regulations and lack of knowledge may be the barriers for hygienic chicken meat production in retail outlets.

IV. CONCLUSIONS

Prevalence of Salmonella in retail chicken meat in Badulla district is higher than the prevalence of E. coli in retail chicken meat in Badulla district. The contamination of E. coli in retail chicken meat due to improper handling and storage of raw chicken meat and poor level of hygienic condition in retail outlets. Undeveloped slaughter facilities, large number of small scale retail businesses, lack of rules and regulations and lack of knowledge are barriers for the hygienic chicken meat production at retail outlets.

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Respiratory Health Problems of Rice Mill Workers in Ampara Divisional Secretariat Division

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Abstract — Rice milling is one of the major occupations in Ampara district which is done in various production scales. Cough, phlegm, chest tightness and wheeze were common among rice mill workers.

A descriptive cross sectional study was conducted to determine the prevalence of respiratory symptoms and lung function measurements of rice mill workers in the Divisional Secretariat Division of Ampara. The participants in the study and control groups were matched for age, height, weight and ethnicity. Prevalence of chronic respiratory symptoms was obtained by a validated questionnaire together with lung function measurements. Forced vital capacity (FVC), forced expiratory volume in the first second (FEV1) and ratio of FEV1 compared to FVC were measured using mini-vitalograph spirometer and peak expiratory flow rate (PEFR) was measured using peak expiratory flow meter. Breathing difficulty, wheezing and having cough were significantly high among the study group compared to the control group (p<0.05) and mean FVC and PEFR were significantly lower among the study group compared to the controls (p<0.01). Therefore, it may be concluded that dust originating from the rice mills causes increased prevalence of respiratory symptoms leading to mixed type of restrictive and obstructive respiratory diseases.

Keywords — rice mill workers, occupational health, lung function measurements

I. INTRODUCTION

Sri Lanka is a paddy growing country from ancient times. North-Central and Eastern provinces are the major paddy cultivating areas although it is done in most of the other parts of the country too.

Ampara district is one of the major paddy cultivating areas in Eastern province of Sri Lanka. As it is situated in dry zone paddy cultivation occur in both seasons, namely, in Yala and Maha with the help of irrigation facilities. Ampara district contributes to 14% (611,244 kg) of the total paddy production in Sri Lanka (Department of Census and Statistics, 2013a & b). After harvesting paddy it is prepared to its two major consumable forms; namely raw rice and parboiled rice by milling process. Raw rice is prepared by milling paddy as it is. To prepare parboiled rice in Sri Lanka it is soaked in water, then either boiled or steamed and then dried well before milling (Abeysekara, 1993; Sumanaweera, 1998; Batsungneon and Kulworawanichpong, 2011). The rice milling process is done in the rice mills using various machineries such as de-huskers, separators, de-stoners, polishers, graders and boilers. Once the paddy is cleaned, it is de-husked, de-stoned and polished.

The milling process being dusty, the mill workers are at a high risk of getting respiratory diseases (Abeysekara, 1993; Desai and Ghosh, 2003; Lim et al 2012; Sumanaweera, 1998). Most of the workers do not use protective equipment or any other form of protective gear although they may be aware of its hazardous outcomes. Therefore, this study attempted to determine the prevalence of respiratory health problems and to compare them with the general community residing from the same
area which will establish a baseline to implement necessary preventive measures.

II. MATERIALS AND METHODS

Ethical clearance was obtained from the Ethics Review Committee of the Faculty of Medicine, University of Colombo. Informed written consent was obtained from all the participants of the study. The study period was between March and April 2014. The study area was Ampara Divisional Secretariat Division (DS division), situated in Eastern Province, Sri Lanka.

A. Study Population

Study group (SG) consisted of all rice mill workers who have been employed for at least four months in each year for more than 3 consecutive years in the rice milling industry in the Ampara DS division. The control group (CG) was selected from the community residing from a randomly selected Grama Niladhari division in the same DS division. They were matched for age (±5 years), height (nearest 1cm), weight (nearest 1kg) and ethnicity on a frequency basis with the study subjects.

A total of 38 rice mill workers for the SG and 30 subjects from the community for the CG were eligible to participate in the study.

B. Study Instruments

British Medical Research Councils (BMRC) questionnaire on Respiratory Symptoms was used to obtain demographic data, personal data and information on respiratory symptoms such as breathing difficulty, wheezing, cough and phlegm.

The BMRC questionnaire on respiratory symptoms was translated to Sinhala and Tamil languages by two experts in the field and back translation was done to ensure the uniformity of the questionnaire with its English version. Content validity of the BMRC questionnaire was assessed to ensure its suitability to the Sri Lankan context with regard to suitability of the wording to the local context and appropriateness of the content to assess respiratory function in Sri Lankan rice millers. This was be done by a panel of 5 experts in the fields of community medicine, occupational medicine and respiratory physicians in clinical medicine and modified accordingly as recommended by them to suit the local context.

The following definitions of respiratory symptoms were adopted:

Chronic cough/phlegm: cough and/or phlegm production on most days in at least 3 months per year.

Breathing difficulty: Shortness of breath when walking with other people at an ordinary pace or their own on level ground.

Chronic bronchitis: presence of cough and phlegm production for a minimum of 3 months a year for at least 2 consecutive years (Jayawardana & Udupihille, 1997).

Wheezing: high pitched whistling sound during breathing (National Institute of Health, 2014).

The mini-vitalograph spirometer used in the field was validated against a more accurate spirometer available in the laboratory of the Department of Community Medicine, Faculty Medicine, University of Colombo. Daily calibration checks of the vitalograph spirometers were done according to American Thoracic Society Guidelines (Miller et al, 2005).

C. Analysis of Data

Socio-demographic characteristics of the study participants and those of the control group were analyzed using descriptive statistics. Control group was matched for age, height, weight and ethnicity with the study group on a frequency basis to ensure that the two groups are comparable.

Prevalence of identified respiratory conditions was calculated for both study and control groups and Chi-square test was used to compare the prevalence of respiratory symptoms in the two groups. Mean values of lung function parameters (VC, FVC, FEV1 and PEFR) were compared between the study and the control groups using independent samples t-test.

III. RESULTS

Mean ages of the SG and CG were 43.95±9.14 (±1SD) and 44.27±9.35 (±1SD) years. Mean height of the two groups were 166.39±4.75 cm (±1SD) and 167±3.91 cm (±1SD) respectively. Mean weight were 66.2±5.1 kg (±1SD) and 66.5±5.2 kg (±1SD) for the SG and CG respectively. Above
differences were statistically not significant (p>0.05). Also the two groups were comparable on age, height, weight and other demographic characteristics (p>0.05) on frequency basis (see Table 1).

The prevalence of the respiratory symptoms in the two groups is given in Table 2 where the study group had higher prevalence of breathing difficulty, cough and wheezing than the control group (p<0.05) although having phlegm and chronic bronchitis were slightly higher among the control group (see Table 2).

Mean values of the FVC and PEFR were significantly lower in the SG than the CG (p<0.01) (see Table 3). However, FEV₁ and FEV₁ ratio were not significantly different between the two groups.

IV. DISCUSSION

This study describes the possible respiratory health hazards and the lung function status of rice milling population in Ampara Divisional Secretariat division. Since the preliminary survey conducted by the principal author had showed a negligible numbers of females who fulfilled inclusion and exclusion criteria, only the male workers were considered for the study group and the control group was selected accordingly. Prevalence of breathing difficulty, chronic cough and chronic phlegm was higher than the findings of the study done in rice mill workers by Abeysekara (1993) which were 15.5%, 11% and 9.1% respectively. However, Wickramage et al (2010) had found a higher prevalence of chronic cough (42%) and chronic phlegm (55%) while wheezing was slightly lower (37%) in mill workers. The less sample size in the current study compared to other

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>SG (n=38)</th>
<th>CG (n=30)</th>
<th>Total No.</th>
<th>Significance (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 40</td>
<td>13 (65)</td>
<td>7 (45)</td>
<td>20</td>
<td>(\chi^2 = 1.907); d.f. = 2</td>
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<tr>
<td>41-50</td>
<td>14 (47)</td>
<td>16 (53)</td>
<td>30</td>
<td>p = 0.385</td>
</tr>
<tr>
<td>More than 51</td>
<td>11 (61)</td>
<td>7 (39)</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>38</td>
<td>30</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td><strong>Income (Rs.)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 19,999</td>
<td>14 (74)</td>
<td>5 (26)</td>
<td>19</td>
<td>(\chi^2 = 5.563;) d.f. = 2</td>
</tr>
<tr>
<td>20,000-29,999</td>
<td>20 (45)</td>
<td>24 (55)</td>
<td>44</td>
<td>p = 0.062</td>
</tr>
<tr>
<td>More than 30,000</td>
<td>4 (80)</td>
<td>1 (20)</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>38</td>
<td>30</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td><strong>Education level</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1-5</td>
<td>26 (59)</td>
<td>18 (41)</td>
<td>44</td>
<td>(\chi^2 = 0.521;) d.f. = 1</td>
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<tr>
<td>Grade 6-O/L</td>
<td>12 (50)</td>
<td>12 (50)</td>
<td>24</td>
<td>p = 0.471</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>38</td>
<td>30</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td><strong>Height (cm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 165</td>
<td>15 (58)</td>
<td>11 (42)</td>
<td>26</td>
<td>(\chi^2 = 0.075;) d.f. = 2</td>
</tr>
<tr>
<td>166-170</td>
<td>15 (54)</td>
<td>12 (46)</td>
<td>27</td>
<td>p = 0.963</td>
</tr>
<tr>
<td>More than 171</td>
<td>8 (53)</td>
<td>7 (47)</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>38</td>
<td>30</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 65</td>
<td>16 (59)</td>
<td>11 (41)</td>
<td>27</td>
<td>(\chi^2 = 0.312;) d.f. = 2</td>
</tr>
<tr>
<td>66-70</td>
<td>14 (52)</td>
<td>13 (48)</td>
<td>27</td>
<td>p = 0.856</td>
</tr>
<tr>
<td>More than 71</td>
<td>8 (57)</td>
<td>6 (43)</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>38</td>
<td>30</td>
<td>38</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Prevalence of respiratory symptoms/conditions in the study group and the control group

<table>
<thead>
<tr>
<th>Symptom/Condition</th>
<th>SG (n=38) No. (%)</th>
<th>CG (n=30) No. (%)</th>
<th>Significance (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dyspnoea</td>
<td>15 (39)</td>
<td>3 (10)</td>
<td>0.006*</td>
</tr>
<tr>
<td>Wheezing</td>
<td>15 (39)</td>
<td>3 (10)</td>
<td>0.006*</td>
</tr>
<tr>
<td>Chronic cough</td>
<td>14 (37)</td>
<td>3 (10)</td>
<td>0.011**</td>
</tr>
<tr>
<td>Chronic phlegm</td>
<td>7 (18)</td>
<td>6 (20)</td>
<td>0.869</td>
</tr>
<tr>
<td>Chronic bronchitis</td>
<td>1 (2.6)</td>
<td>1 (3.3)</td>
<td>0.865</td>
</tr>
</tbody>
</table>

* Significant at p<0.01  **Significant at p<0.05

Table 3. Comparison of mean lung function measurements between the study group and the control group

<table>
<thead>
<tr>
<th>Lung function measurement</th>
<th>SG (n=38) Mean ±SD</th>
<th>CG (n=30) Mean ±SD</th>
<th>Significance (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVC (L)</td>
<td>2.68 ±0.36</td>
<td>2.91 ±0.32</td>
<td>0.006*</td>
</tr>
<tr>
<td>FEV₁ (L)</td>
<td>2.1 ±0.39</td>
<td>2.25 ±0.28</td>
<td>0.069</td>
</tr>
<tr>
<td>FEV₁ ratio</td>
<td>78.1 ±8.3</td>
<td>77.4 ±5.4</td>
<td>0.689</td>
</tr>
<tr>
<td>PEFR (L/min)</td>
<td>386 ±62.83</td>
<td>425 ±34</td>
<td>0.003*</td>
</tr>
</tbody>
</table>

* Significant at p<0.01

The higher prevalence of respiratory symptoms in the study group than the control group found in the current study may be due to irritant effects of rice husk dust exposure together with allergic responses either due to protein constituent of rice husk or microbiological contaminant (Lim et al, 1984; Dhillion & Kaur, 2011).

Significant decline in FVC in the rice mill workers found in current study was parallel with Dhillion and Kaur (2011) which is suggestive of restrictive type of lung impairment due to changes in bronchi and elastic component of lungs (Dhillion and Kaur, 2011). Significant decrease of PEFR compared to the control group may be caused by hypertrophy of mucosal cells due to irritation by grain dust and smoke resulting in the increased secretion of mucus and formation of mucosal plugs which cause obstruction to the exhaled air (Dhillion and Kaur, 2011). However the other lung functions were not significantly differ from the control group.

The cross sectional nature of the current study prevented assessing temporal associations. Also this study was unable to highlight a dose-response relationship however, it is emphasized that other factors affecting the results should also be considered.

Considering all the factors, it is concluded that rice mill workers are at a risk of acquiring respiratory symptoms and lung impairments. Therefore, it is necessary to implement preventive measures by means of controlling dust emission, educating the workers regarding the risks and possible outcomes and the importance of using preventive measures.

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REFERENCES


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Hobsen’s Choice in Disaster Medical Ethics

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Abstract— Medical ethics is founded on three basic principles; which are the principles of beneficence, non-maleficence and respect for autonomy. The priority of these principles may change with different circumstances, such as in disasters, which sometimes may lead to challenges that are quite different from day today medical practices. Disasters make the medical practitioner more vulnerable to hazards; the hazardous working environment causes extraordinary additional stresses that a practitioner may not undergo in a normal environment.

Disasters may lead to ethical challenges that are different from usual medical practices. In addition, disaster situations are related to public health ethics more than medical ethics, and accordingly may require stronger effort to achieve a balance between individual and collective rights. The author researched extensively on ethical consideration of Disaster Medicine. This paper aims to review some ethical dilemmas that arise in disasters and mainly focuses on health services.

Disasters vary considerably with respect to their time, place and extent. Therefore, ethical questions may not always have 'one-size-fits-all' answers. On the other hand, embedding ethical values and principles in every aspect of health-care is of vital importance. It is only by making efforts before disasters, that ethical challenges can be minimized in disaster responses.

Keywords—Hazard, Ethical dilemma, ethical challenges, disaster medicine

I. INTRODUCTION

The disasters can be identified as natural, man-made or a combination of both when considering the aetiology of the disaster. This is better because it allows the responding organization to tailor its response. Another way of understanding disaster is whether disaster occurred due to internal or external factors. A good example for internal disaster is bomb threat to a hospital or blast in the hospital. An external disaster for the hospital is a blast in the community hall which makes the hospital, the response organization.

Disasters, whether unintentional acts of nature or human-made, can have profound effects on those who experience them. Although the physical dangers inherent in disasters are obvious, such events, including terrorism and bioterrorism, are a grave threat to mental health as well. Previous episodes of disaster have dramatically affected individuals, communities, and nations. In addition to affecting physical and mental health acutely, disasters can have more chronic impacts creating social and economic hardship, loss of employment, the dissolution of personal relationships, and the long-term deterioration of physical and mental health.

Complicating the situation is the absence of a standard definition of disaster, much less a uniform concept for an academic discipline of disaster medicine. The need to codify this emerging discipline and create such standards is becoming increasingly clear. The specialty of emergency medicine evolved as a result of the recognition of the special considerations in emergency patient care, and similarly the recognition of the unique principles in disaster related public health and medicine merit the establishment of their own formal discipline. Such a discipline will provide a foundation for doctrine, education, training, and research and will integrate preparedness into the public health and medical communities.

Ethics is the study of standards of conduct and moral judgment of a system or code of morals. Medical ethics is a system of moral principles that apply values and judgments to the practice of medicine. Modern medical ethics is founded on three basic principles; which are the principles of
beneficence, non-maleficence and respect for autonomy.

A. Beneficence
The term beneficence refers to actions that promote the wellbeing of others. In the medical context, this means taking actions that serve the best interests of patients. However, uncertainty surrounds the precise definition of which practices do in fact help patients. James Childress and Tom Beauchamp in their book Principle of Biomedical Ethics (1978) identify beneficence as one of the core values of healthcare ethics.

B. Non-Maleficence
The concept of non-maleficence is embodied by the phrase, “first, do no harm,” or the Latin, primum non nocere. Many consider that should be the main or primary consideration (hence primum): that it is more important not to harm your patient, than to do them good.

C. Autonomy
The principle of autonomy recognizes the rights of individuals to self-determination. This is rooted in society’s respect for individuals’ ability to make informed decisions about personal matters. Autonomy has become more important as social values have shifted to define medical quality in terms of outcomes that are important to the patient rather than medical professionals. Respect for autonomy is the basis for informed consent and advance directives.

II. DISASTERS AND ITS MANAGEMENT CYCLE

Disaster Management is the discipline dealing with and avoiding risks. It is a discipline that involves preparing, supporting and rebuilding society when natural or human-made disasters occur. We can divide any disaster situation in to three phases.

A. Pre disaster Phase - Mainly involves the mitigation that tries to minimize the effects of disaster. Good Examples are building codes and zoning; vulnerability analyses; public education. Preparedness is planning how to respond. Some Examples are preparedness plans; emergency exercises/training; warning systems etc.

B. Disaster Phase - Response is efforts to minimize the hazards created by a disaster.

C. Post disaster Phase – Mainly involve recovery which is basically returning the community to normal with some development. Good examples are temporary housing to permanent housing; grants; medical care, infrastructure development in better ways etc.

III. ETHICS IN DISASTER MEDICINE

Ethics in disaster medicine deals with ethical issues and dilemmas in natural and human-made disasters. Disaster ethics is a very broad field, in a sense that it compasses numerous topics from individual to collective ethics. We will discuss disaster ethics addressed in three phases of disaster: (i) pre-disaster (pre-event) or preventive phase, ii) disaster (event/ crisis) and early response phase, and iii) post-disaster (post-event) or rehabilitation phase.

A. Ethics in Pre-Disaster Phase
Developing strategies to prevent disasters or to decrease the magnitude of the disaster related injuries and damages are regarded as an ethical responsibility. Developing a preventive ethics approach in this pre-disaster phase also helps to reduce conflicts during the crisis phase. Within this scope, capacity building to increase knowledge and skills of disaster relief professionals and the populations at risk, developing disaster recovery
plans, practicing and updating these plans as needed, building strong partnerships among organizations and institutions with potential duties in disaster relief, preparing legislations and manuals as to better respond to the ethical conflicts in disasters as well as informing all partners about this ethical framework are crucial. Accordingly, the World Medical Association (WMA) recommends that disaster medicine training be included in the curricula of university and post-graduate courses in medicine.

B. Ethics in Disaster and Early Response Phase

In this phase, reaching the disaster site as quickly as possible is the most crucial step. In line with the principles of the ethical practice of public health, “Public health institutions should act in a timely manner on the information they have within the resources and the mandate given to them by the public”. If the health authorities and health-care workers act slowly, ignoring the fact that time is vital, they may be late in saving lives and violate the principle of doing no harm.

Triage, as the second most important step, is often considered critical in the distribution of limited medical resources, where highest priority should be given to the principles of beneficence and justice. According to the WMA Statement on Medical Ethics in the Event of Disasters (1994); “In selecting the patients who may be saved, the physician should consider only their medical status, and should exclude any other consideration based on non-medical criteria”. Many systems (e.g. START, SIEVE, Homebush Triage etc.), have been proposed for the so-called primary triage or scene triage, which is defined as the initial assessment of victims at the disaster site.

WMA recommends that the physicians should set an order of priorities for treatment that will save the greatest number of lives and restrict morbidity to a minimum. In connection with this, some patients, whose condition exceeds the available resources, may be classified as "beyond emergency care". According to WMA (1994), “It is ethical for a physician not to persist, in treating individuals "beyond emergency care". The physician must show such patients compassion and respect for their dignity, hence separate them from others and administer appropriate analgesia sedatives. Halpern and Larkin (2006) contribute to this discussion by stating that health care has to be equitably distributed rather than equally, with each victim receiving care according to medical need. On the other hand, critical questions on when and how to apply disaster triage still remain. Domres B; Kock M; Manger A; Becker HD; (2001) argue that disaster triage is ethical only under extreme situations. Sztajnkrycer MD; Madsen BE; Bae'ez A; (2006) also contribute to this discussion by reporting that resources are rarely scarce in events viewed by the general public as disasters and the number of affected people may be misleading to switch to disaster triage.

In literature, there are numerous studies that reveal high rate of over-triage and under-triage in disaster situations. WMA recommends that disaster triage should be entrusted to authorized, experienced physicians, assisted by a competent staff.

Informed consent, which is used frequently on daily medical practice, is another important ethical challenge in disasters. There might be exceptions to informed consent, such as in disaster and other public health emergency situations. WMA Statement on Medical Ethics in the Event of Disasters (1994) states that in a disaster response, it should be recognized that there may not be enough time for informed consent to be a realistic possibility. Although health professionals in disaster relief are expected to make every effort to start and sustain available treatments according to priority, some victims might refuse treatment. In that situation, mental health state of the victim should be assessed. If there is any doubt, the treatment should be continued to avoid any medical or legal consequences. If the examination reveals no significant mental problem, then health professionals may try to convince the person for the recommended treatment, whenever possible. If time permits, the last option might be to ask the victim to sign a document indicating that s/he does not accept the treatment.

Refusal of treatment might be more complex in pandemic disasters. Although refusal of treatment usually seems as an individual decision, the patient’s right to refuse treatment may conflict with the health professionals’ duty to protect public health. Patients with highly contagious diseases may pose a significant threat for other people. In these situations, every effort should be made for
the diagnosis and proper treatment of infected persons, including trying to convince the patients, who refuse treatment, by explaining the potential risks to public health.

Pandemic disasters may also pose other ethical dilemmas with respect to the autonomy of individuals. Although the WMA International Code of Medical Ethics (1949) states that “A physician shall owe his/her patients complete loyalty”, it is generally accepted that physicians may in exceptional situations have to place the interests of others above those of the patient. Mandatory reporting of patients who suffer from designated diseases is one such exception. Physicians should fulfil their duty to report, although patients should be informed that such reporting will take place. Public health measures in pandemic disasters, such as vaccination campaigns, risk communication, quarantine and isolation are also worth noting with respect to potential ethical dilemmas. In all of these dual-loyalty situations, protection of public from harm is usually regarded as a superior goal than respect for autonomy. In the case of vaccination, it is widely accepted that risk-benefit ratios must be calculated for all immunizing agents.

According to Last (2004), scientists working in emergency situations like an epidemic, have an ethical duty to be open in dealing with the public. Last argues that public has the right to know what the experts know. Within this scope, implementing the principles of risk communication to avoid unnecessary fear and anxiety among the public is of vital importance. In disaster situations, delivery of appropriate and updated information to health-care workers on a regular basis is also critical to minimize misinformation, mistrust and refusal of public health measures among the public. According to Soliman and Rogge (2002), information helps survivors make informed decisions that are intrinsically related to their life arrangements and future well-being.

WMA Declaration of Lisbon on the Rights of the Patient (1981) states the major role of physicians in allocation of scarce resources as the following: “In circumstances where a choice must be made between potential patients for a particular treatment that is in limited supply, all such patients are entitled to a fair selection procedure for that treatment. That choice must be based on medical criteria and made without discrimination”.

Development of clinical practice guidelines in the pre-disaster phase and using these guideline-based criteria in health resource allocation in the response phase may minimize potential ethical conflicts that arise during decision-making in disasters. In this regard, Lin and Anderson-Shaw (2009) proposed a clinical model for decision making in allocation of mechanical ventilators in disaster/pandemic situations, which was based on the ethical principles of beneficence and justice and utilized the concept of triage. In the model, the researchers recommended formation of a pandemic triage committee to allow decisions to be made by a team of professionals rather than individual physicians. In addition, they proposed a palliative care protocol and early family involvement for families of patients to be aware of the protocols, thus avoiding potential conflicts.

The division of labour among organization and institutions is considered as one of the ethical aspects of disaster response. Accordingly, every effort should be made to assign labours according to the expertise of each organization. Hussein (2010) contributes to this issue by stating that networking with other service providers is both an ethical and an operational need. Spending available financial resources, as another ethical issue in disaster response, should also be considered. According to the UNDP (1997), millions of dollars are spent on salaries, per diems, transportation, and other costs for the disaster relief experts of countries other than the affected country; however, disaster response spending should primarily be done by contracting services from the affected communities themselves.

Health workforce is one of the most important human resources in disaster response. On the other hand, this workforce might be negatively affected by disaster conditions (e.g. pandemic outbreaks, environmental pollution, military conflicts), which may pose significant threats for the relief workers’ own safety and health. Hesitation of health-care workers to perform their duty in pandemic disasters is one such example. Many studies in literature indicate that health professionals constitute a significant proportion of the victims in pandemic situations. In addition, numerous literature findings reveal unwillingness of at least some health professionals’ to treat patients with communicable diseases. In daily practice, medical codes of ethics make no exception for infectious patients with
regard to the physician’s duty to treat all patients equally; however, disaster conditions might have its own unique risks. Therefore, the question of where to draw the line between acceptable and unacceptable level of risk for health-care workers still remains to have more concrete answers.

According to a recent WHO publication (2007) on ethical considerations in pandemic influenza; “Countries should develop policies that clearly delineate health-care workers’ obligations, which can be recognized in one or more of the following ways: moral obligations, professional obligations, contractual obligations, non-contractual legal obligations.” Furthermore, it is stated that the duty of health-care workers to work with health risks is not unlimited. The guide concludes that “From an ethical perspective, the least problematic enforcement mechanisms are those that have been voluntarily adopted by those who will be affected by them. Thus, governments should encourage professional organizations to develop policies regarding professionals’ obligations to work during epidemic”. WHO (2007) also recommends governments and employers to minimize risks to health-care workers by giving adequate education and by taking preventive measures, which the health-care workers, by an ethical obligation are expected to comply with. In case of morbidity or mortality of health-care workers, medical and social benefit systems are proposed. According to the UNDP (1997), relief institutions have special ethical obligations to their staff during humanitarian emergencies. Adequate preparation and training beforehand, and effective counselling and support during and after operations are strongly advised.

In literature, complex humanitarian emergencies and armed conflicts are often classified as disasters. In relation with the duty of health professionals in emergency and disaster situations, participation in the acts of torture, death penalty or inappropriate treatment constitute a critical ethical challenge. WMA, with the Declaration of Hamburg Concerning Support for Medical Doctors Refusing to Participate in, or to Condone, the Use of Torture or Other Forms of Cruel, Inhuman or Degrading Treatment (1997), is responsible to support physicians who resist to be involved in such inhuman procedures or who work to treat and rehabilitate victims.

Respect for diverse values, beliefs, and cultures in the community constitute one of the principles of the ethical practice of public health. Disasters generally create situations, in which, some health services are delivered by health-care workers who are originally not from the affected area. Foreign health-care workers, whether from the affected country or from another country may have difficulties in communicating with the patients or treating them.

Besides interfering with optimum health care, cultural, religious and linguistic barriers may also have significance with respect to creating ethical dilemmas. If health professionals and patients do not speak the same language, every effort should be made to find interpreters. However, in the presence of cultural or religious differences, interpreters may not be enough to overcome communication problems. Without any preparation, international relief workers may be at risk for delivering culturally inappropriate services, such as distribution of condoms to adolescents of a conservative community. Such interventions might negatively affect the overall relief efforts. Therefore, foreign professionals have an ethical duty to be aware of any cultural and religious differences, when delivering preventive and curative health services. According to the WMA Statement on Medical Ethics in the Event of Disasters (1994); the physician must respect the customs, rites and religions of the patients. In this respect, community participation in disaster relief efforts is a useful approach in planning services, which are ethically sound and widely accepted by the affected community. Ensuring an opportunity for input from community members is also one of the principles of the ethical practice of public health. This approach helps to deliver services on a needs-based. Most studies in literature indicate that participation of community members make significant changes in the recovery phase of disasters.

Another principle of the ethical practice of public health is the empowerment of disadvantaged community members. Health professionals working in disaster response should pay special attention to vulnerable groups; including children, women, elderly, people with disability, refugees and other minority groups, since these groups are usually affected more negatively than the general population. In line with the ethical principle of justice, it is also crucial for relief workers to try to
avoid actions that may cause stigmatization and discrimination of vulnerable groups.
Vulnerability is also related with the topic of disaster research, which is among the most important ethical challenges’ of disaster medicine. Today, there are numerous policy documents, such as WMA Declaration of Helsinki (1964), International Ethical Guidelines for Biomedical Research Involving Human Subjects (2002) and The Ethics of Research Related to Healthcare in Developing Countries (2002) that guide health professionals in their scientific research; however, disaster situations may pose their own conflicts with respect to the study group, informed consent, etc. That’s why research in disasters may be difficult to perform according to the existing declarations. The event and early response phase of disaster, there is greatest respondent vulnerability and least social order to conduct research. In the long term recovery period, the social order increases, whereas the vulnerability of affected people decreases. According to Abramson (2007), the social context of a disaster defines the research and the ethical landscape. In relation with this context, he proposes researchers to ask several critical questions before conducting any disaster research: timing of the study, presence of any time limit, nature of exposure to disaster or agent, characteristics and vulnerability of the study group, potential risks and benefits to study participants, local support, informed consent, referrals for help, logistics and potential risks to the research team are the main questions that have to be answered by the researchers to plan disaster studies in the most appropriate and ethical way. According to the principles of the ethical practice of public health, public health institutions should protect the confidentiality of information that can bring harm to an individual or community. However, media interest in the disasters and people affected by disasters raises ethical issues on privacy and the principle of respect for autonomy.

Media plays an important role in dissemination of information for both the general community and disaster victims. In addition, disasters covered by the media receive more attention. However, media news may interfere with the private life of the victims. In addition, the WMA Statement on Medical Ethics in the Event of Disasters (1994) states that the physician has a duty to each patient to ensure confidentiality when dealing with third parties. It is also important to designate health-care workers, who are experienced in media relations. Another ethical issue with media relations is that some organizations tend to work in disaster relief primarily for the media coverage, since they link future funding options with their image in the media. Here, the ethically appropriate approach would be to provide assistance with the primary and only goal to help disaster victims, which will eventually be followed by positive responses from both donors and the public in general.

C. Ethics in Post-Disaster Phase

All ethical values and principles that were mentioned for pre-disaster and early response phases should also be recognized in the aftermath of disasters; however, health care professionals should act according to the requirements of the new phase. WMA Statement on Medical Ethics in the Event of Disasters (1994) states that “In the post-disaster period, the needs of survivors must be considered. Many may have lost family members and may be suffering psychological distress. The dignity of survivors and their families must be respected”.

According to the UNDP (1997), a disaster response should prevent future disasters and decrease vulnerability of the victims. Opposite to common thinking, developmental efforts should start with the early stages of disaster response, rather than the post-disaster phase. This approach avoids development of a dependency syndrome among the people affected by the disaster. Participation of the community to disaster response does not only help to determine priority needs of and culturally appropriate interventions for the affected community, but also helps to actively engage people to work for their community’s own rehabilitation and development.

Hussein (2010) argues that dependence on foreign aid, directing financial resources to NGO’s rather than the local health infrastructure, shift of the local health-care workers to international NGOs, where they are better paid and inappropriate division of labour, where similar health services are delivered by different relief organizations delay the development process in the affected community. Therefore, starting with the earliest possible time in disaster response, efforts should be made to build the infrastructure of the healthcare system, with the community’s own human resources for health.
IV. SRI LANKAN SCENARIO

The author had the privilege to treat patients in the biggest two disasters that our country faced, the Asian Tsunami and humanitarian war. During the Tsunami Author worked disaster and post disaster situations. Humanitarian war the author worked in all three phases of disaster. Although both situations are unique on its’ own context similarities too observed. In disaster phase the amount and extent of injuries in casualties needed triage and rapid evacuation gave no opportunity for informed consent. But there were no evidence for breach of confidentiality or ill treatment. Since military is a well-organized institution usually they were the first responders in any disaster that the country faced. They utilized scarce resources including man power and available meagre air assets for aids and aid group transportation, medical team transportation, and casualty/medical evacuation extremely well.

During the Asian Tsunami with the influx of medical aids we observed some expired drugs some luxuries items and military type equipment such as dismantled helicopters too came with medical aids. Different medical teams from different countries had their own agendas and protocols. Lack of command and control over different teams caused problems of accumulation of medical aids to some places and some affected area were neglected. There were some allegations of collection of blood samples from patients without informing them the reasons for collection of blood and also giving injections without informing reasons for giving injections against their wish in post tsunami rehabilitation period.

Some aids organizations were more sympathetic towards some ethnic groups caused immense damage to ethnic harmony of the country.

V. CONCLUSION

Disasters vary considerably with respect to their time, place and extent; therefore, ethical questions in these situations may not always have ‘one-size-fits-all’ answers. On the other hand, embedding ethical values and principles in every aspect of health-care is of vital importance in disasters. For the very reason; reviewing legal and organizational regulations, developing health-care related guidelines, protocols and disaster recovery plans by taking potential ethical dilemmas into account, establishing on-call ethics committees as well as adequate in-service training of health-care workers for ethical competence are among the most critical steps to take in pre-disaster phase. These measures should be taken both at the local level as well as the country level. In conclusion, it is only by making great efforts before disasters, that ethical challenges can be minimized in disaster responses.

LIST OF REFERENCES


BIOGRAPHY OF AUTHOR

Author is a Wing Commander from the Air Force, PhD student and presently works as Base Dental Officer at Air Force Base Ratmalana. His research interest includes aviation dentistry, tactical casualty care, ethics & disaster medicine. He is a disaster reliance leadership fellow and has produced over 12 publications in local and international publications to his credit. He is involved in teaching disaster medicine to post graduate students in his leisure time.
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