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Editorial

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GALLE MEDICAL JOURNAL; INSTRUCTIONS TO AUTHORS

The Galle Medical Journal is published by the Galle Medical Association. The *journal* is published biannually, March and September and the submissions are accepted throughout the year. The aims of the journal are to foster co-operation among the medical fraternity and to be a forum to make literary contributions, share experiences encountered in medical practice, update their knowledge and have debates on topics related to all aspects of medicine. Also, we attempt to cater to the educational needs especially of the postgraduate trainees. The *Journal* publishes original articles, reviews, leading articles and case reports. When an article is submitted for publication, we expect that the work it reports has not been published, submitted simultaneously to another journal or accepted for publication elsewhere. All manuscripts will be reviewed anonymously before acceptance.

Manuscripts must be submitted with the text type written in 12-point Times New Roman font double spaced. Text and all illustrative material should be submitted in two hard copies and the electronic version in Microsoft Word document format. In order to avoid delay we require authors to comply with the following requirements. **All manuscripts should accompany a covering letter indicating the number of words in the manuscript, institution where ethical clearance was granted, conflict of interests and contact details of the corresponding author.**

Types of contributions:

Review articles and Leading articles: We encourage submission of review or leading articles which are less than 3000 words in length and address topics of current interest. They should be supported by no more than 20 references. Submissions may be subjected to external review before acceptance.

Original articles: Should normally be in the format of introduction, methods, results and discussion. Each manuscript must have a structured abstract of 200 words. The text should be limited to 3000 words and maximum of 5 tables/figures taken together with no more than 15 references. Lengthy manuscripts are likely to be returned for shortening. The discussion in particular should be clear, concise and should be limited to matters arising directly from the results. Avoid discursive speculation.

Case Reports: These should not exceed 750 words and 5 references; no abstract is required. Case report should be informative and devoid of irrelevant details. Case report should have a clear message or learning point and this should be highlighted adequately. Rarity of the case does not mean it is suitable for publication. Written consent of the patient should be submitted together with the case report, especially when photographs are used.

References:
These should conform to the Vancouver style. The reference in the text should be numbered consecutively in Arabic numerals in parentheses in the same line of the text in the order in which they appear. The first five authors should be listed and if there are more than five, then the first three should be listed followed by et al. Examples are given below:

1. Kumar A, Patton DJ, Friedrich MG. The emerging clinical role of cardiovascular magnetic resonance imaging. *Canadian Journal of Cardiology* 2010; **26**(6): 313-22.

2. Calenoff L, Rogers L. Esophageal complication of surgery and lifesaving procedures. In: Meyers M, Ghahremani G, eds. Iatrogenic Gastrointestinal Complications. New York: Springer, 1981: 23-63.

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Manuscripts that do not conform to these requirements will be returned for necessary modifications.

Manuscripts should be addressed to Chief Editors, Galle Medical Association, Teaching Hospital, Karapitiya and all soft copies should be sent to *gmathk@gmail.com*

Tissue micro array; establishing a cost-effective tool for cancer biomarker research in Sri Lanka

Running title: TMA for cancer biomarker research in Sri Lanka

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ABSTRACT

Tissue micro array is a cost effective tool for cancer biomarker discovery and for the validation and external quality control in immunohistochemistry. It has not been utilized in Sri Lanka before, although widely used in cancer research centres world over. Scarcity of cancer biomarker research in Sri Lanka is partly due to the unaffordable cost of laboratory consumables including antibodies. TMA is produced using tissue cores from multiple tissue blocks. It reduces the cost and improves the consistency in immunostaining and adds validity to the assessment. In this brief report, we describe the technique of producing TMA and technical issues faced and how we could overcome them.

Key words: *TMA, cancer biomarkers*

Review

The concept of embedding tissue of different samples into one tissue block goes back to 1986 when the 'sausage' tissue blocks were developed for immunohistochemical assessment (1). The advantage of sausage block was that all of the tissue samples are treated equally during immunostaining and most sources of variation are eliminated which facilitates comparative studies. It was recommended for large scale inter-laboratory quality control processes. This concept was further developed and Tissue Micro Array (TMA) was designed to its current format by Kononen *et al* in 1998 (2). Now it is an invaluable research tool in cancer biomarker discovery.

TMAs are paraffin wax blocks (recipient blocks) constructed with tissue cores extracted from multiple tissue wax blocks (donor blocks). TMAs are sectioned and histology slides are prepared and can be stained with any routine histological stains and immunohistochemistry. It is a high-throughput technology useful in histology based laboratory tests and can be used in florescent in situ hybridization as well (3). TMA can also be used to assess molecular parameters (DNA, RNA) by molecular techniques. While TMA is made, a template per block is prepared indicating the reference number to map the clinical details of the patient with the biomarker score. Once made, TMAs can be used for subsequent assessment of multiple markers. Therefore, TMAs can be used as tissue libraries for future research. TMA cuts down on the cost for antibodies and reagents by many folds as a small core of representative tissue is carefully selected instead of a routine tissue sample. The selected size of the core can be 0.6 to 2 mm. Therefore, a TMA block can be built with hundreds of tissue cores minimizing the variation that can occur during staining procedures improving the validity and increasing the cost-effectiveness. In this brief report, we intend to describe our experience in how this technique can be established in a routine histopathology laboratory.

TMA blocks can be constructed manually or by using precision instruments. Automated forms of tissue micro arrayers are also available but less cost effective for a country like ours. In our histopathology laboratory we used a TMA Builder (Thermo Fisher™) and manually constructed TMAs for a research project on immunohistochemical biomarker assay for a cohort of breast cancer patients who's clinic-pathological and survival details were available for mapping and subsequent analysis.

Making a TMA block

The TMA Builder consists of a mould and a punch extractor. The base of the mould has 24 pins which makes 24 pits in the recipient block. The mould-top has an inset for C-ring and two lifting screws (Figure 1).

Paraffin wax pellets were melted at 60 °C in an oven to bring to liquid state. The base of the TMA mould was placed on a flat surface and a plastic C-ring was fitted into the inset in the mould-top. Molten wax was poured slowly to fill the C-ring which was then left to solidify. Once wax was solid enough, the C-ring filled with wax was removed from the metal mould by screwing down the two lifting-screws. The prepared wax block now has 24 pits to receive 24 tissue cores.

The donor tissue blocks were first examined for its physical suitability. The haematoxylin and eosin (H&E) stained slides of each case were reviewed. The best representative tumour region with minimum fixation artifacts was selected and marked for tissue extraction. The slide was superimposed on the corresponding donor block to identify the area in the tissue to be punched.

From each of these donor blocks, a core of 2 mm diameter tissue was extracted using the punch extractor of the TMA Builder™ (Thermo Fisher). The cores were transposed/injected into the pits in the recipient TMA wax mould prepared previously.

A core of brain tissue from a wax block was transposed into the 24th pit in the mould as a guide to identify the rows and the columns of the TMA. A template for each TMA block was prepared to link the biomarker score to clinico-pathological data of each case. We made 53 such TMA blocks containing breast cancer tissue of 1200 patients. Since the diameter of each tissue core was 2 mm which covers a sufficient surface area, tissue cores were not taken in duplicate (4).

TMA block was labeled in accordance with the template and was kept in the oven with the wax surface with tissue cores facing down on a flat metal surface. Temperature was set to 58 °C and left for 15 minutes to anneal the block and to bring the tissue cores to the cutting surface. Sections were cut at 4µ on a traditional microtome. Slides were kept overnight in the incubator at 60 °C before staining was done. Sections were assessed by light microscopy. TMAs also can be digitally scanned and displayed on a high resolution monitor (4). Scoring of the biomarkers on TMA was done blinded to the clinico-pathological data reducing the potential for bias. We were able to link this data to survival outcome and were able to prove the prognostic significance of immunohistochemical assessment of biomarkers in breast cancer which included new biomarkers (5). There are no other published reports on TMA technique being used for cancer research in Sri Lanka.

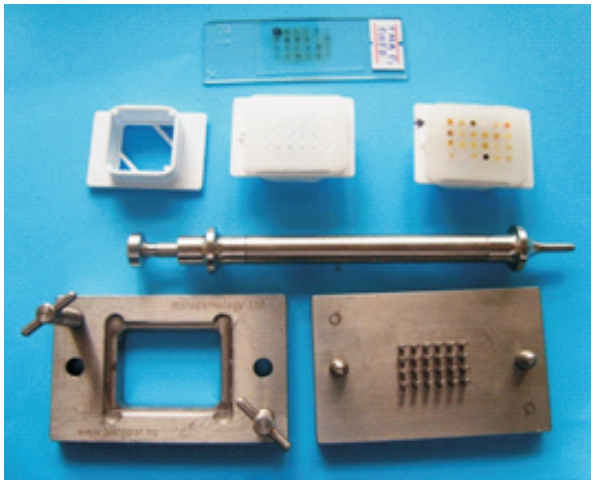

Figure1: This shows (items from top to bottom and from right to left) 1. A TMA slide stained for EGFR antigen; black circle indicating the guide core, 2.C-ring, 3. A recipient TMA paraffin block with 24 pits built on a C-ring, 4. A TMA block containing 24 tissue cores, 5. Punch extractor, 6. Mould-top of the TMA Builder with inset for C-ring and two lifting-screws in place, 7. Base of the TMA Builder with 24 pins.

Problems identified / troubleshooting

1. Locating the correct area to extract in a core biopsy donor block was difficult. The shape of the core of tissue which appeared on the block and matching it with the corresponding slide was used as a guide.
2. Since it is important to leave some diagnostic material in the block as archive, many core biopsies had to be excluded from our study. This was a limiting factor in preparing TMAs from core biopsies.
3. The depth to which the punch should cut into the donor block has to be first determined by trial as the extracted cores should be of the same length to fit into the pits in the recipient blocks.
4. Some tissue blocks were already sectioned extensively for the diagnostic process leaving only a thin piece of tissue and wax. The cores obtained from such blocks were very short compared to the depth of the TMA pit. The problem of shorter cores not reaching the cutting surface of the recipient block was resolved by keeping the TMA blocks in an oven as described in the annealing process. However, tissue loss was observed as such cores wore off after a few sections were obtained.
5. When the tissue in the recipient block contains fat around the tumour, correct superimposing of the slide to mark the correct site for core extraction was difficult. Inked resection margins, if available, were of help in such situations.
6. Breaking off of the outermost column of the tissue cores was frequently observed when the TMAs were sectioned. This occurred when the TMA blocks were fixed to the microtome through the plastic wings of the C-ring. The problem of breaking of blocks was overcome by fixing the TMA block through the frame of the C-ring.
7. Applying ice cubes on the surface of the TMA block just prior to sectioning, further reduced breakage of blocks.
8. Overnight incubation of tissue sections at 60 ^{o}C in the hot air oven, prior to immuno-histochemical staining, minimized the loss of tissue cores. This does not replace the necessity for a good section adhesive or a charged slide. Loss of tissue cores was minimal with H&E staining. When the guide core of the tissue section was lost during immunohistochemical staining, the H&E stained slide was very useful in identifying the location of the guide core. Therefore, it is advised to have the first section to be stained with H&E.
9. Folding of tissue sections was encountered at times. This occurred when the sections were too thick. Good microtomy skills were of utmost importance in obtaining sections of correct thickness without folds or cracks.

We believe that the information given in this brief report will be of value for Sri Lankan Histopathologists who wish to research into tumour biology and validate biomarkers using the plethora of cancer specimens they report and to find new knowledge on biomarkers at an affordable cost. It will be also useful in establishing external quality control system for immunohistochemistry laboratories in the country, which is a long-felt need.

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Compliance with Ethical Standards

This research project was granted approval from the Ethical Review Committee of the Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka.

Conflicts of interest

Author SNG received a monthly stipend as research assistant from the funding authority. The other authors declare that they have no conflicts of interest. The funding agency had no involvement in the study other than providing sufficient funds to conduct the research.

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Socioeconomic status and stress: Neuroendocrine pathways to disease

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ABSTRACT

The biomedical model investigates diseases of the organism at the cellular level, and ignores the broader socioeconomic factors that affect health. This discursive analysis examines the physiological mechanism by which socioeconomic factors get *inside* the human body and make people susceptible to diseases. A survey of recent materials was carried out using PubMed database to identify peer-reviewed manuscripts that examine physiological reaction to stress and its connection to diseases. These studies reveal a complex relationship between socioeconomic status and chronic diseases.

Key words: *Stress, adaptation, allostasis, allostatic load, illness*

Introduction

During the last three decades, researchers have been able to establish the physiological connection between socioeconomic status and diseases such as hypertension, coronary heart diseases, stroke, type II diabetes, memory impairment, and malignancies, which seem to increase as we go down the socioeconomic ladder of a society. While the social gradient may explain the continuous distribution of risks and vulnerabilities, the fundamental question that these researchers have been trying to answer was how does socioeconomic status get *inside* our bodies and make us susceptible to diseases? This discursive analysis, based on the current literature, explains the physiological mechanism through which socioeconomic status manifests as a major cause of disease, and makes some policy recommendations to mitigate the socioeconomic challenges, and their health damaging consequences.

Understanding the Social Determinants of Health:

The social determinants of health include a wide range of interrelated socioeconomic factors that affect people's everyday living. For example, income and employment status, the level of education, early childhood experience, and access to healthcare are some of the important social and economic factors that affect health throughout life. Yet, almost universally, these factors are not adequately incorporated into health policies and practices. Health has long been regarded as an outcome of good medicine. Although modern medical breakthroughs have eradicated many infectious diseases, the level of *exposure* to disease risks is often dependent upon a wide range of socioeconomic factors. It is only recently that policy makers have begun to examine population health within the broader context of socioeconomic status, and to introduce population health promotion strategies as part of the overall socioeconomic policies.

The social and economic problems influence health in every stage of life. For example, recent studies have shown that low birth weight and emotional deprivation in childhood are major causes of learning disabilities and other behavioral problems, which could in turn be a precursor to long-term material disadvantages in adult life. Moreover, these

material disadvantages are both absolute and relative: for example, not having money to buy food, or a place to live is a measure of an absolute poverty, which is a common problem among the people of the lowest rung of the social hierarchy. The effect of absolute poverty during in-utero growth through early childhood is known to produce biological markers for many adulthood diseases, such as diabetes and coronary artery diseases (1).

Relative poverty, on the other hand, is the difference between the standard of living enjoyed by the people in upper social echelons and those in the lower social classes in the same society. Relative poverty is believed to affect health through psychological and other status symbols. Although absolute poverty has significantly declined in recent years, relative poverty is rising throughout the world due to widening income gap between the rich and the poor. Studies have shown that in societies where socioeconomic disparities are higher, the overall mortality rate, infant mortality, violent crimes, hostility, obesity, and interpersonal distrust are greater (2). Thus, health is powerfully affected by both absolute and relative socioeconomic status. As we go down the social ladder within a given society, morbidity and mortality rates increase because the same people suffer from both types of deprivation. How does socioeconomic status get "under the skin," and make people sick?

Adaptation to Socioeconomic Pressure:

The link between socioeconomic status and disease is "stress," the term that often used to express the physical and psychological "pressure" exerted by various external challenges. These external challenges, which include socioeconomic problems, are powerful stressors in life. However, the impact of these stressors varies depending on the length of exposure to such challenges, the availability of supportive resources, and the individual genetic factors. Bruce McEwen defines stress as "a threat, real or implied, to the psychological or physiological integrity of an individual" (3). He recognizes, however, the ambiguity of the term as it is used in everyday life, which makes it difficult for us to understand how the human body copes with stress. As researchers have pointed out in recent years, this coping mechanism, which is described as *allostasis,* enables the organism to adapt to the external challenges, so that it can maintain the internal functional balance (*homeostasis*) of organ systems, and sustain life. Any physical or psychological challenge disturbs the functional balance of organ systems, and the body reestablishes it by adjusting neuroendocrine adaptation. Humans have survived on the earth by adapting to their physical and social environments. Overtime, however, the coping mechanism increases the vulnerability to major diseases, which is described as the *allostatic load*, or the cumulative wear and tear on the organ systems and their tissues due to prolong, insufficient, or failed adaptation (4).

As the brain determines the nature of the challenge, it regulates neuroendocrine response. Coping with any acute challenge requires extra energy for physical and psychological endurance. Thus, the autonomic nervous system, via the hypothalamic-pituitary-adrenal (HPA) axis, triggers the *catabolic* function of the digestive system resulting in an increase release of energy by breaking down protein, carbohydrate, and fat storages in the muscles and the liver. The release of three major endocrines (hormones), such as *epinephrine*, *norepinephrine,* and *dopamine* (collectively known as *catecholamine*) from the adrenal medulla increases the supply of blood to the heart, brain, and skeletal muscles. This in turn triggers fundamental physiological reactions affecting blood pressure: 1) the release of *epinephrine* triggers the discharge of the *glucagon* hormone by the pancreas to breakdown *glycogen,* the energy storages in the liver, which increases the plasma levels of glucose, free fatty acids, low-density lipoprotein, and cholesterol to provide more energy; 2) the discharge of *norepinephrine* stimulates the heart muscles to increase cardiac output (the heartbeat), and vasoconstrictions (tightening of the arteries) to eject more blood to the brain and skeletal muscles, and less blood to the gut and kidney; 3) *norepinephrine* stimulates the release of *renin-aldosterone* hormone in the kidney, which increases the plasma sodium concentration and arterial blood pressure. The *renin-aldosterone* mechanism increases the retention of sodium fluids in the blood flow, and prevents releasing the liquid through urine; 4) *dopamine*, primarily a neurotransmitter from the brain and autonomic nervous system, regulates the blood flow through the arteries, and the secretion of both *epinephrine* and *norepinephrine.* With the rising cardiac output, fluid

volume, and vasoconstriction, the blood pressure rapidly elevates forcing more blood to the brain and skeletal muscles, while restricting the blood flow to elsewhere in the body (5).

As these neuroendocrine mechanisms intensify the functional output of several organ systems, the release of the *glucocorticoid* hormone from the adrenal cortex regulates the *catabolic* process that increases the blood level of glucose. Obviously, all these physiological and neuroendocrine reactions to an external challenge require a fundamental shift in the performance of a number of major organ systems from an *anabolic* state of repairing tissues, producing immune cells, stimulating reproductive hormones, and storing excess energy to a *catabolic* state in which more energy is provided to certain organ systems to intensify their activity, while suppressing the performance of others temporarily in order to sustain life (or to face the challenge).

Adaptation and Disease:

The stress induced disease, or *allostatic load,* is the chronic wear and tear on the body resulting from adaptation to stressor. As noted, a common outcome of the adaptation (*allostasis*) is cardiovascular reactivity resulting in high blood pressure. The elevated blood pressure accompanies several risk factors such as increased blood levels of glucose, low-density lipoprotein, and cholesterol, which are all known clinical risk factors for heart diseases, diabetes, obesity, and strokes. Studies have shown that once the neuroendocrine process raises the blood pressure, and maintains it at a higher level for an extended period, blood pressure tends to stay high even if the initial cause of the elevation no longer exists. The brain and arteries develop structural and functional remodelling as part of the adaptation. This remodelling, over time, particularly in connection with arterial constriction, causes damage in the inner lumen of the arterial walls, where atherosclerotic plaques begin to develop, obstructing the blood flow. At this stage, even if the fluid volume has become normal, the narrowed arteries with blockages are bound to cause heart attacks and strokes (6).

Also, the release of *glucocorticoid* hormone during stress undermines the production of white blood cells, which has several pathological consequences. During prolonged stress the suppressed immune system delays the healing of wounds and injuries, and most importantly exposes the organism to various pathogenic agents such as viruses, bacteria, and carcinogenic agents, which are normally removed from the body by white blood cells. The immune system, particularly the thymus gland and its ability to produce white blood cells, is mediated by *glucocorticoids* (7).

Likewise, under chronic stress, *glucocorticoid* impedes the insulin function that regulates the blood glucose level. The function of insulin is to remove the extra glucose from the blood and store them in the muscles, body fat, and the liver as *glycogen* to be utilized when the blood glucose level goes down. During stress, the increased demand for energy to meet an external challenge prevents this insulin activity resulting in abnormally high levels of blood glucose. While elevating blood levels of glucose and free fatty acids, and preventing insulin from storing them in the muscles and the liver, the *glucocorticoid* promotes the deposition of *glycogen* in the abdomen. It has been identified that abdominal obesity, as in Cushing's disease, as well as type I and II diabetes are triggered by chronic stress (8). A number of recent studies have substantiated these observations reporting that excessive metabolic disorders among low-income groups, such as African-Americans, are related to psychosocial stress of living in hierarchical societies (9). Kate Pickett and colleagues found that in the top 50 developed countries, adjusting for gross national per capita income, the distribution of income is *positively* correlated with a number of health indicators such as the percentage of obese people in the population, diabetes-related mortality rate, and the average daily per capita calories intake (10).

While the brain is the main organ that regulates the neuroendocrine response to stress, it is also subjected to major neurological remodeling and structural transformations due to prolonged stress. Both animal and human studies have shown that prolonged activation of *glucocorticoid* receptors of the brain increases the risk of protein plaques deposition on the neurons obstructing the chemical messengers (neurotransmitters). The increased blood flow to the brain also carries with them high levels of plasma protein that accumulates on the neurons over time, eventually destroying them altogether. The *amyloid hypothesis* suggests that the accumulation of *amyloid* plaques on neurons may be linked to Alzheimer's disease. Recent studies of type II

diabetes, which is connected to increased plasma level of *glucocorticoid* under chronic stress, found reduced hippocampal volume (shrinking of the hippocampus). They have also shown that the reduced hippocampal volume is associated with memory impairment and cognitive dysfunction, and a general relationship between the size of the hippocampus and spatial memory (11). *Studies based on* human neuroimaging have shown that prolonged stress and post-traumatic stress disorders produce drastic structural changes in the prefrontal cortex, hippocampus, and amygdala leading to chronic depressive symptoms and anxiety disorders (12).

The neuroendocrine reactions triggered by socioeconomic challenges inevitably impose undue pressure on the organ systems and their tissues in the long-term. Generally, it is constructive when the neuroendocrine reactivity is rapidly mobilized on the organ systems and terminated immediately. However, when the neuroendocrine reactivity is prolonged due to chronic stress, it undermines mental and physical health. How rapidly those neuroendocrine mechanisms are activated and are halted is dependent upon the availability of effective coping mechanisms and resources for individuals, families, and communities. Unhealthy coping mechanisms could further aggravate damaging health consequences.

Behavioural Adaptations as Coping Mechanisms:

The pathophysiological conditions resulting from the adaptation to stress are often exacerbated by a variety of harmful behavioural adaptations, such as smoking, drinking, overindulging, and the lack of physical activity, which are often perceived as coping strategies by some individuals. These behavioural adaptations have the same pathogenic outcomes for major organ systems, as do the physiological adaptions to stress.

By contrast, however, studies have shown that the availability of social support, trusting interpersonal relations, regular physical activity, and membership in community organizations, which have been collectively described as *social capital*, enhances the resilience of organ systems. Any supportive relationship that has an attenuating effect on psychophysiological stress may have a positive long-term impact on health (13). People with social networks receive both material and emotional support during stressful situations in their lives. Social connectivity and supportive environments are known to increase inhibitory signals to the HPA axis, which in turn raises the HPA activation threshold thereby minimizing the effect of potential socioeconomic challenges to physiological system. In other words, if the individual is confident that support is available at a time of "distress" such knowledge could prevent the full-blown stress response from the HPA axis. The individual differences in social integration and connectivity (social networks) could therefore modify the cognitive perceptions of external challenges, which may in turn moderate the neuroendocrine reactions to such challenges (14).

Conclusion: Policy Recommendations

In recent years, particularly in developed Western countries, public policies on population health promotion have increasingly been incorporated into major economic policies. There has been significant effort to reduce the social gradient in health through inclusive economic policies, which have the potential to increase prosperity, greater redistribution of resources, and the overall social cohesiveness. These policies are results of several well-recognized public inquiries into the impact of socioeconomic inequalities in some countries, and the global campaigns by various multilateral organizations, such as the World Health Organization (WHO) and the World Bank, to inform about the "health cost" of inequality and economic marginalization. For example, the report of the Donald Acheson Commission of the United Kingdom, and the WHO Commission on Social Determinants of Health advocate that population health strategies to be based on four major pillars to counter the potential impact of material disadvantages from early childhood through adulthood: 1) reducing poverty and income inequality through targeted redistributive welfare policies to give greater educational and employment opportunities for disadvantaged social groups; 2) preventing unhealthy life-styles and life course issues, such as smoking, drinking, and malnutrition to promote healthy development; 3) prohibiting discrimination based on age, gender and ethnicity in areas such as employment, education and housing to

ensure social justice and equality; 4) ensuring universal access to quality healthcare, particularly primary care (15).

The underlying philosophy that guides these broad policy recommendations is that good health for all can be achieved by providing a good "foundation" in life through guaranteed access to education, healthcare and employment. Life is a progression through stages, in that material disadvantages in early childhood that prevent getting a good education, undermines the labour market opportunities during adulthood. People who have experienced disadvantages in early childhood are at the greatest risk in the subsequent stages of life. Policies need to prevent people from experiencing disadvantages at the earliest possible stages of life, and they need to give priority to those who are already at a disadvantage in society. These are the people who suffer from both absolute and relative deprivations.

The social environments–at work, school, or neighbourhood–that provide a sense of belongingness, security and safety are known as "healthy environments" as they prevent marginalization and deprivation, and promote a sense of well-being. In such social environments, individuals are less likely to make unhealthy lifestyle choices. By contrast, environments, in which people feel excluded, abused and discriminated, are regarded as "toxic environments," where people feel less worthy of themselves, seek the comfort of health damaging behaviours, and engage in criminal activities. Therefore, national and local authorities, as well as public and private employers, and community leaders need to recognize the health implication of social integration in institutions, as well as, in communities and neighbourhoods. Policies that prevent discrimination, abuse and violence not only increase economic productivity, but also promote health and well-being. People who are more socially connected: 1) live longer; 2) are more likely to survive a myocardial infarction; 3) are less likely to experience a recurrence of cancer; 4) are less likely to suffer from infectious illness, than those who are less integrated to the community (16).

Likewise, access to healthcare is a basic human right that has been recognized by the World Health Organization. Specifically, the access to primary care is critical for preventing disease, immunizing children and providing prenatal care services that build the foundation for healthy living. Although health care services themselves focus on the clinical aspects of risks rather than the social determinants of exposure to risks, preventive health services protect people from diseases and premature death. Public policies must ensure that all citizens have guaranteed access to essential health services, so that an unforeseen illness would not prevent people from fully participating in education and employment resulting in life-long socioeconomic disadvantage. Public policies need to address the socioeconomic determinants of health before they manifest as major health problems. This is the challenge for both policy makers and political leaders.

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Exploring the pleiotropic effects of vitamin D in diabetes

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Introduction

Vitamin D is a unique vitamin and a hormone which is absolutely essential for bone mineral homeostasis. In addition, in the recent past there has been a growing appreciation for its role as an endocrine regulator having important roles in the renal, cardiovascular, reproductive and immune systems in the body. These actions are named non-classic actions or pleiotropic effects of vitamin D.

Regarding the non-classic actions of the hormonal form of vitamin Din the renal system, inhibition of Renin Angiotensin System (RAS)by reducing renin synthesis is an important action of vitamin D . Renin is the first and the rate limiting step of the RAS cascade.

This action of vitamin D can be used to overcome the unwanted effects resulting from the long term use of Angiotensin Converting Enzyme Inhibitors (ACEI) and Angiotensin Receptor Blockers (ARB) in patients with renal and cardiovascular disease; that is compensatory rise in renin level as a result of disruption of feedback inhibition of renin production.

ACEI and ARB are included in the standard treatment for reducing proteinuria in diabetic nephropathy, which is the commonest cause of end stage renal disease worldwide . Despite the treatment with ACEI and ARB, rate of progression of diabetic nephropathy remains high. Compensatory rise in plasma renin level leading to activation of RAS is one important mechanism which has been postulated as a possible reason for the inadequacy of ACEI and ARBs on reversing diabetic nephropathy.

Current data on the role of vitamin D reducing the progression of renal damage in diabetic renal disease are limited and come from either animal studies or few human studies . Studies which explored the effect of vitamin D on animal models of kidney disease have reported the beneficial effects of such therapy. Randomized control trials examining the effect of vitamin D on the progression of proteinuria are limited ,. Further, these studies are not sufficiently powered to generate conclusive results. There is a paucity of sufficiently powered randomized controlled trials examining the different reno-protective effects of vitamin D among patients with diabetic nephropathy.

Hence we planned to evaluate the effect of vitamin D therapy in reversing the progression of diabetic nephropathy and also to determine the effect on plasma renin, cardiovascular disease risk (CVDR) profile, bone mineral density (BMD) and bone mineral content (BMC) measurements.

This review includes the following arms exploring the effects of vitamin D on

1. renal functions in patients with diabetic nephropathy.
2. lipid profile and blood pressure in patients with diabetic nephropathy.
3. cardiovascular risk scores in patients with diabetic nephropathy.
4. bone mineral density in patients with diabetic nephropathy.

Methods

This study was conducted on patients with diabetic nephropathy (urinary albumin >30 mg/g of creatinine in two occasions) whose GFR was more

than 30 mL/min. Patients who had albuminuria in a previous cross sectional study six months back were invited and investigations were repeated. This procedure ensured confirmation of albuminuria at least on two occasions over a period 6 months. Selected patients were informed about the study and written consent was obtained. Those who had blood pressure > 130/80 mmHg during the last two clinic visits, hyperphosphataemia (serum phosphate > 5 mg/dL), hyper or hypocalcaemia, uncontrolled blood sugar (current HbA1c > 8) and those with liver disease, hyperthyroidism, hyperparathyroidism, or diseases related to calcium or vitamin D metabolism and congestive heart failure (current) were excluded. Attempts were made to exclude other causes of proteinuria such as ongoing urinary tract infection, urolithiasis, and renal tuberculosis by history, examination and previous investigations. Morning urine samples were collected and urine dipstick test was done to exclude ongoing urine infections. Urine collection was postponed if a patient had fever, urinary symptoms, or menstruation. Only those were negative for nitrates were stored in -80^{0}C for urine albumin analysis. After two weeks second sample of urine was collected. Same procedure was followed as for the first urine sample. If the urinary albumin excretion of the second sample was inconsistent with the first sample, a third urine sample was checked. Presence of microalbuminuria was confirmed only if two consecutive or two out of three samples were positive for urine albumin > 30 mg albumin/g of creatinine. In the same manner, macroalbuminuria was confirmed when urine albumin excretion was > 300 mg/g of creatinine.

Study design

Patients were allocated to two groups by block randomization method (block of 2) using a random number table. Concealed envelopes containing treatment allocation were given to research assistants who assigned participants to treatment and control groups. Treatment group received monthly dose of 50,000 IU of vitamin D_3 intramuscularly and the control group was given an equal volume of distilled water (0.25 mL) to the same site in a similar manner. Participants, those administering the interventions, clinicians, and those assessing the outcomes were blinded to the group assignment.

Study Procedures

Patients underwent a detailed medical history, a physical examination including systolic and diastolic blood pressure (SBP and DBP) measurement. Blood and urine were collected for the baseline measurements which included serum creatinine, serum calcium, urine microalbumin, fasting blood glucose (FBS), serum calcium, phosphate, creatinine, Parathyroid Hormone (PTH), renin and vitamin D level and lipids namely total cholesterol (TC), low density lipoprotein (LDL), triglycerides (TG), and high density lipoprotein (HDL). CVDR was calculated using Framingham risk score (FRS). The CVDR, fatal coronary vascular disease risk (FCVDR), stroke risk (SR) and fatal stroke risk (FSR) were obtained by the UKPDS (United Kingdom Prospective Diabetes Study) risk engine.

A safety visit was scheduled 1 week after starting the treatment to monitor the serum Ca and phosphorus concentrations and to elicit any adverse events. The protocol permitted withdrawal from the trial if serum Ca exceeded 11 mg/dL. During monthly visits patients were inquired about side effects according to a check list in the data collection sheet.

All patients underwent whole body dual-energy X-ray absorptiometry (DXA) scan and BMD and BMC of the total body, total spine (L_1-L_4) and proximal femur were measured. All scans were performed and analysed by the same technician adhering to the manufacturer's protocol. DXA machine was calibrated using the calibration phantom provided by the manufacturer. The precision error of the machine has been published previously . There were no software or hardware changes during the study period.

At three months blood samples were collected for serum creatinine, serum calcium, serum phosphate, FBS, lipid profile and urine was taken for the assessment of urine micro albumin to creatinine ratio at three months.

After six months of treatment all the measurements done at the baseline, including DXA were repeated. When the trial period of six months was over, a randomly selected subgroup of patients (25 from each group) was followed up for further six months and another DXA testing was performed.

Biochemical assays were performed using commercial kits. Urine albumin was measured by turbid metric method while urinary creatinine concentration was measured using an end-point spectrophotometric method with an alkaline-picrate solution.

Serum creatinine was measured by auto-creatinine calibrated with autocal which is traceable to reference material. Glomerular Filtration Rate (GFR) was estimated by CKD-EPI equation.

Intact PTH (Immunotech, IRMA PTH), renin (Beckman coulter, IRMA Active Renin) by radioimmunoassay and 25-hydroxy vitamin D were measured using immunochemiluminometric (Vitros immunodiagnostic) assays. Serum creatinine was measured by spectrophotometric method with an alkaline-picrate solution.

Ethical aspect

Ethical clearance was obtained from the Ethics Review Committee of the Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka.

Clinical trial has been registered in the local clinical trial registry. Informed written consent was obtained from all subjects of the study.

Statistical analysis

The baseline characteristics between the two groups were compared by either unpaired t-test or Chi-square test. Changes in urinary albumin, renal functions, vitamin D, renin, PTH, BMD/BMC during the trial period were analyzed by the Repeated measure ANOVA (SPSS, Chicago, USA). P value was adjusted for multiple comparisons by the Bonferroni method.

Results

A total of 157 patients were invited for the study and 72 were excluded due to the presence of one or more exclusion criteria. Remaining 85 were randomly assigned to two groups and 82 subjects completed the study; 41 patients from each group completed the study.

No significant differences were found with regards to the baseline characteristics between the treatment and control groups (Table 1).

Table 1: Baseline characteristics of subjects in the two groups

Variable	Control group (n = 43)	Treatment group(n = 42)	*P* value
Age (years)	59 (8)	56 (10)	0.1
Number of males (%)	42.9	48.8	0.58
SBP (mmHg)	121 (7)	120 (8)	0.46
DBP (mmHg)	70 (5.9)	71 (5.9)	0.25
HbA_1C (%)	7.1 (0.5)	6.9 (0.5)	0.1
Calcium (mg/dL)	8.9 (0.7)	8.8 (0.6)	0.65
Phosphorus (mg/dL)	3.8 (0.6)	3.9 (0.5)	0.31
PTH (pg/mL)	42.5 (19.0)	38.2 (11.3)	0.21
Plasma renin (pg/mL)	15.14 (4.82)	14.64 (5.62)	0.66
25(OH)D (nmol/L)	49.6 (16.5)	56.1 (12.9)	0.07
FBS (mg/dL)	130 (12.5)	128 (13.3)	0.51
Duration of diabetes (years)	7 (4)	8 (5)	0.42
Urine creatinine (mg/dL)	63.6 (10.9)	61.7 (11.9)	0.44
Urine albumin (mg/g of creatinine)	185.8 (50.6)	164.4 (35.8)	0.83
Glomerular Filtration Rate (mL/min)	83.2 (16.1)	86.7 (14.6)	0.28
HDL (mg/dL)	53.5 (10.9)	50.3 (7.5)	0.13
TC (mg/dL)	194.6 (32.1)	194.8 (30.1)	0.87
LDL (mg/dL)	117.0 (28.1)	119.7 (28.7)	0.87
TG (mg/dL)	128.4 (50.8)	122.8 (41.4)	0.66
BMI (kg/m^2)	23.2 (4.0)	24.4 (3.4)	0.14

SBP (systolic blood pressure), DBP (diastolic blood pressure), PTH (parathyroid hormone), FBS (fasting blood sugar), HDL (high density lipoprotein), TC (total cholesterol), LDL (low density lipoprotein), TG (triglyceride), BMI (body mass index)

All patients received either an ARB or ACEI at the baseline. During the study period, oral hypoglycemic drugs were increased in nine patients (6 in the treatment group). Losartan was increased in three patients (2 in the control group). Blood pressure number and doses of anti-hypertensive drugs were quite stable during the clinical trial.

1. Vitamin D therapy on renal functions of patients with diabetic nephropathy

Diabetic nephropathy is the leading cause of end stage renal disease and despite optimum therapy including ACEI/ARBs, a sizable proportion of patients with proteinuria progress to renal failure. It is likely that high renin level induced by RAS (Renin Angiotensin System) blockage may contribute to this and vitamin D is found to have an inhibitory effect over RAS as it reduces renin synthesis.

This study was conducted to examine the effects of vitamin D therapy on renal functions of patients with diabetic nephropathy.

Effect of vitamin D therapy on urinary albumin excretion, renal functions and plasma renin among patients with diabetic nephropathy; a randomized, double-blind clinical trial.

Results

Table 2 shows the changes of the urine microalbumin to creatinine ratio, systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting blood sugar (FBS), serum creatinine, eGFR, PTH, renin and vitamin D levels after three and six months of treatment in the treatment and control groups. After six months, mean reduction of urinary albumin to creatinine ratio was 51.8 mg/g ($P = < 0.001$) in the treatment group, 22.4 mg/g ($P = 0.06$) in the control group and this difference was significant ($P = 0.001$). Significant increase in the GFR was observed in the treatment group while in the control group GFR remained unchanged ($P = 0.03$ for the between-groups difference). There was a significant reduction of serum creatinine in the treatment group but not in the control group. But the change was not significant between groups.

A significant increase of SBP was seen in the control group whereas SBP remained unchanged in the treatment group and the difference was not statistically significant. Significant trends in the DBP was seen in both groups during the study period but the difference between the two groups was not statistically significant ($P = 0.17$). Significant reduction of FBS was seen only in the control group and the difference between groups was not statistically significant ($P = 0.23$).

Significant reduction of PTH was observed in both treatment and the control groups. But the change between two groups was not statistically significant ($P = 0.26$). In the treatment group, vitamin D level increased by 25.64 nmol/L and between the two groups the change was statistically significant ($P < 0.001$). Mean reduction in plasma renin in the treatment group was 5.85 pg/mL ($P < 0.001$). In the control group the reduction observed was only 0.95 pg/mL. The difference between the two groups was statistically significant ($P = 0.006$) (Table 2).

A significant inverse correlation was observed in vitamin D with percentage change in plasma renin level ($\rho = -0.66$, $P < 0.01$) and percentage change in urine albumin levels ($r = -0.47$, $P < 0.01$). Furthermore, percentage changes of renin and urinary albumin also showed a significant correlation ($\rho = 0.62$, $P < 0.01$) (Table 3).

According to the table 4, the microalbuminuria suppression is related to the final serum renin level. The suppression of microalbuminuria is highest in the highest tertile of serum renin and it does not vary across the tertiles of serum vitamin D. The lowest suppression of microalbuminuria is in the lowest tertile of the serum renin. Furthermore, there is a gradient of microalbuminuria suppression across the tertiles of the serum renin concentration.

Serum Ca in patients with vitamin D treated and in the control group were 9.16 (0.61) and 9.045 (0.69) at the end of trial. The difference was not statistically significant. No adverse events, particularly hypercalcaemia were reported during the study period.

Table 2: Changes observed in the treatment and control groups at 3 months and 6 months

Variable		Baseline	At 3 months	At 6 months	*P* within group	*P* between group
SBP (mmHg)	Control	121 (7)	121 (8)	127 (6)	< 0.001	0.07
	Treatment	120 (8)	120 (8)	121 (7)	0.59	
DBP (mmHg)	Control	70 (6)	72 (6)	72 (6)	< 0.001	0.17
	Treatment	71 (6)	69 (6)	68 (6)	< 0.001	
FBS (mg/dL)	Control	130.2 (12.5)	130.6 (10.1)	127.8 (10.7)	0.02	0.23
	Treatment	128.3 (13.6)	125.8 (13.4)	125.9 (10.9)	0.08	
PTH (pg/mL)	Control	42.5 (19.0)		37.6 (12.6)	0.003	0.26
	Treatment	38.2 (11.3)		35.7 (7.9)	0.001	
25 (OH)D (nmol/L)	Control	49.64 (16.46)		45.67 (17.20)	0.004	< 0.001
	Treatment	56.11 (12.95)		81.75 (15.03)	< 0.001	
Plasma renin (pg/mL)	Control	15.14 (4.82)		14.19 (4.6)	0.02	0.006
	Treatment	14.64 (5.62)		8.83 (4.81)	< 0.001	
Urine albumin (mg/g)	Control	185.8 (50.6)	160.9 (63.4)	163.4 (56.2)	0.06	0.001
	Treatment	169.4 (35.8)	122.1 (54.4)	117.6 (45.2)	< 0.001	
Serumcreatinine (mg/dL)	Control	0.87 (0.22)	0.87 (0.20)	0.87 (0.20)	0.84	0.10
	Treatment	0.86 (0.13)	0.80 (0.12)	0.77 (0.11)	< 0.001	
GFR (mL/min)	Control	83.2 (16.1)	83.4 (15.6)	83.9 (14.9)	0.74	0.03
	Treatment	86.7 (14.6)	90.7 (14.8)	93.7 (14.1)	< 0.001	

SBP (systolic blood pressure), DBP (diastolic blood pressure), PTH (parathyroid hormone), FBS (fasting blood sugar), GFR (glomerular filtration rate)

Table 3: Correlations (Spearman rho) between the percentage change in vitamin D, urine albumin, plasma renin and PTH

Percentage change	Urine albumin	Renin	PTH
Vitamin D	-0.47**	-0.66**	-0.02
Urine albumin		0.62**	-0.08
Renin			-0.02

**Correlations are significant at 0.01 level.

Table 4: Percentage change in urinary albumin excretion in relation to of vitamin D and renin

Vitamin D ↓	Renin → Low	Middle	High
Low	32.9 (18.3)	11.9 (7.4)	5.7 (9.1)
Middle	45.0 (8.1)	10.0 (11.6)	2.9 (8.6)
High	33.5 (6.9)	12.6 (11.6)	5.8 (8.6)

Values are given mean (SE)

Conclusions

Randomized double-blind placebo controlled clinical trial conducted among patients with diabetic nephropathy showed a significant reduction of urine microalbumin, serum creatinine, renin levels and improvement of GFR among patients in the treatment group compared to the control group after monthly injection of vitamin D for six months.

2. Vitamin D therapy on lipid profile and blood pressure in patients with diabetic nephropathy

Effects of six month, high-dose parenteral vitamin D therapy on lipid profile and blood pressure in patients with diabetic nephropathy; a randomized double-blind clinical trial

Aim of this study was to determine the effect of high dose vitamin D given to patients with early diabetic renal disease on systolic and diastolic blood pressure, total cholesterol (TC), low-density lipoproteins (LDL), triglycerides (TG) and high density lipoproteins (HDL) in a randomized controlled trial.

Results

Table 5 shows the changes in SBP, DBP, TC, TG, LDL, HDL and vitamin D at 3 and 6 months of follow up.

In the treatment group, vitamin D level increased by 25.64 nmol/L and between the two groups the change was statistically significant (P < 0.001).

Vitamin D therapy significantly reduced DBP, total cholesterol and LDLC in the treatment group, but the between group differences were not significant. There was an increase in HDL cholesterol level in the treatment group while there was no change in the control group (the between groups difference was significant).

Table 5: Changes in CVDR factors in the treatment and control groups

Variable		Baseline	At 3 months	After 6 months	*P* value within group	*P* value between group
SBP	Control	121 (7)	121 (8)	127 (6)	< 0.001	0.07
(mmHg)	Treatment	120 (8)	120 (8)	121 (7)	0.59	
DBP	Control	70 (6)	72 (6)	72 (6)	< 0.001	0.17
(mmHg)	Treatment	71 (6)	69 (6)	68 (6)	< 0.001	
TC	Control	194.6 (32.1)	193.6 (30.8)	196.9 (31.4)	0.24	0.50
(mg/dL)	Treatment	194.8 (30.1)	191.5 (28.1)	185.7 (27.2)	< 0.001	
TG	Control	128.4 (50.8)	127.9 (49.5)	128.7 (45.3)	0.62	0.44
(mg/dL)	Treatment	122.8 (41.4)	121.8 (40.1)	118.2 (32.4)	0.062	
LDL	Control	117.0 (28.1)	114.6 (28.9)	117.1 (30.2)	0.34	0.7
(mg/dL)	Treatment	119.7 (28.7)	115.7 (27.6)	106.10 (26.5)	< 0.001	
HDL	Control	35.5 (10.9)	53.7 (10.7)	53.9 (9.7)	0.40	< 0.001
(mg/dL)	Treatment	50.3 (7.5)	51.5 (7.1)	55.7 (6.8)	< 0.001	
25 (OH)D	Control	49.64 (16.46)		45.67 (17.20)	0.004	< 0.001
(nmol/L)	Treatment	56.11 (12.95)		81.75 (15.03)	< 0.001	

SBP (systolic blood pressure), DBP (diastolic blood pressure), TC (total cholesterol), TG (triglyceride), LDL (low density lipoprotein), HDL (high density lipoprotein)

Conclusions

Randomized double blind placebo control clinical trial conducted among patients with diabetic nephropathy has shown significant improvement in HDL levels but no significant effect on blood pressure after monthly injection of vitamin D for six months.

3. Vitamin D therapy on cardiovascular risk scores of patients with diabetic nephropathy

Effects of six month, high-dose parenteral vitamin D therapy cardiovascular risk scores in patients with diabetic nephropathy; a randomized double-blind clinical trial

Diabetes is considered as a coronary vascular disease equivalent. Cardiovascular disease risk (CVDR) can be assessed by cardiovascular risk scores. The aim of this study was carried out to assess the effects of vitamin D therapy on CVDR scores among patients with diabetic nephropathy.

CVDR was calculated using Framingham risk score (FRS). Further, CVDR, fatal coronary vascular disease risk (FCVDR), stroke risk (SR) and fatal stroke risk (FSR) were obtained by the UKPDS (United Kingdom Prospective Diabetes Study) risk engine.

Results

No significant differences were found between treatment and control groups at the baseline. FRS, CVDR, FCVDR, SR and FSR values before and after the intervention were 20.32 and 21.41, 13.62 and 11.94, 8.97 and 7.951, 7.66 and 7.50, 0.95 and 0.94 respectively in the treatment group. Respective values of the control group were 19.96 and 22.49, 12.76 and 13.59, 7.902 and 8.80, 6.11 and 6.66, 0.75 and 0.87.

No significant effect was found with vitamin D_3 treatment on CVDR scores, measured by FRS and UKPDS (Table 6).

Table 6: Changes in CVD risk scores in the treatment and control groups

Variable		Baseline	At 3 months	At 6 months	*P* value within	*P* value between
FRS	Control	19.96 (11.09)	19.79 (12.02)	22.49 (11.69)	< 0.001	0.92
	Treatment	20.32 (14.14)	19.69 (13.73)	21.41 (21.61)	0.62	
CVDR	Control	12.76 (08.33)	12.72 (08.37)	13.59 (08.69)	.004	0.88
	Treatment	13.62 (11.27)	12.57 (09.85)	11.94 (09.55)	< 0.001	
F CVDR	Control	7.902 (6.32)	8.05 (6.10)	8.80 (6.60)	0.006	0.96
	Treatment	8.97 (9.40)	8.09 (7.82)	7.951 (7.10)	0.005	
Stroke risk	Control	6.11 (4.35)	6.15 (4.40)	6.66 (4.70)	< 0.001	0.51
	Treatment	7.66 (9.60)	6.80 (7.70)	7.50 (9.00)	0.11	
F Stroke risk	Control	0.75 (0.55)	0.76 (0.56)	0.87 (0.62)	< 0.001	0.55

Framingham risk score (FRS), Cardiovascular disease risk (CVDR), fatal coronary vascular disease risk (FCVDR), fatal stroke risk (F Stroke risk)

Conclusions

Monthly injections of high dose vitamin D_3 but did not have a significant effect on cardiovascular risk scores among patients with diabetic nephropathy.

4. Vitamin D therapy on bone mineral density of patients with diabetic nephropathy

Effect of vitamin D therapy on bone mineral density among people with diabetic nephropathy; a randomized, double-blind placebo controlled clinical trial.

Aim was to determine the effect of vitamin D given to patients with diabetic nephropathy on bone mineral density (BMD) and bone mineral content (BMC).

Results

A total of 157 people were invited for the study and 72 were excluded due to the presence of one or more exclusion criteria. Remaining 85 were randomly assigned to two groups; 43 subjects in the treatment group and 42 subjects to the control group. No significant differences were found with regards to the baseline characteristics between the treatment and control groups.

Two participants from the treatment group and one participant from the control group did not complete the study. They were not contactable probably due to the change of the residence. Forty one subjects from each group completed the intervention. At the end of 6 months DXA results were available in 39 participants in the treatment group and 38 in the control group. At the end of one year, 25 from each group underwent the 3rd BMD measurement.

Table 7 shows the changes of the total body BMD/BMC, regional BMDs, total fat and lean masses, during the initial six months of treatment in the treatment and control groups.

After six months of vitamin D injections, total body BMD, total body BMC and BMDs of spine, femoral neck and total hip regions increased by 2.0%, 2.2%, 1.8%, 2.1% and 2.6% ($P < 0.05$ for all), respectively from the baseline figures. However, increase observed in the trochanteric BMD among them was not statistically significant. In the control group, compared to the baseline values, total body BMC, BMD, or regional BMDs did not change significantly during the initial six months.

Table 7: Changes bone mineral density and fat mass in the treatment and control groups

Variable		Baseline	After 6 months	Percentage difference	*P* within groups	*P* between groups
BMD	Control	1.038 (0.121)	1.031 (0.191)	-0.67	0.75	0.61
	Treatment	1.038 (0.120)	1.059 (0.107)	2.02	0.01	
BMC	Control	1775.63 (412.76)	1721.64 (369.70)	-3.04	0.074	0.73
	Treatment	1757.95 (383.68)	1795.85 (373.27)	2.16	0.007	
Spine BMD	Control	0.848 (0.132)	0.836 (0.119)	-1.41	0.27	0.72
	Treatment	0.845 (0.153)	0.860 (0.142)	1.78	0.04	
Femoral neck BMD	Control	0.722 (0.109)	0.712 (0.094)	-1.38	0.23	0.43
	Treatment	0.731 (0.153)	0.746 (0.142)	2.05	0.03	
Trochanter BMD	Control	0.607 (0.089)	0.604 (0.08)	-0.49	0.5	0.46
	Treatment	0.615 (0.111)	0.627 (0.103)	1.95	0.07	
Hip BMD	Control	0.857 (0.113)	0.852 (0.105)	-0.58	0.56	0.25
	Treatment	0.876 (0.148)	0.899 (0.149)	2.62	0.008	
Total fat mass	Control	15.85 (6.67)	16.48 (6.16)	3.99	0.20	0.2
	Treatment	17.41 (5367.460)	18.21 (5.56)	4.6	0.06	
lean mass	Control	37.04 (6.94)	36.98 (6.38)	-0.18	0.86	0.16
	Treatment	38.92 (8.32)	39.64 (7.73)	1.85	0.09	

BMD (bone mineral density), BMC (bone mineral content

Six months after the cessation of vitamin D treatment, significant reductions of both total BMD and BMC were observed (P = 0.009) while regional BMDs remained unchanged (Table 8). In the control group none of the BMD / BMC measurements changed significantly during the post-trial follow up six months period.

Table 8: Changes bone mineral density and fat mass in the treatment and control groups

Variable		After 6 months		After 12 months		Percentage difference	*P* within groups	*P* between groups
BMD	Control	0.999	(0.134)	1.006	(0.112)	0.70	0.47	0.21
	Treatment	1.054	(0.120)	1.041	(0.131)	-1.23	0.009	
BMC	Control	1735.92	(430.22)	1716.05	(402.87)	-1.14	0.26	0.54
	Treatment	1808.19	(450.57)	1795.94	(458.27)	-0.68	0.04	
Spine BMD	Control	0.823	(0.128)	0.828	(0.126)	0.61	0.19	0.69
	Treatment	0.847	(0.168)	0.837	(0.163)	-1.18	0.07	
Femoral neck BMD	Control	0.711	(0.109)	0.711	(0.105)	0	0.92	0.28
	Treatment	0.756	(0.166)	0.753	(0.169)	-0.4	0.48	
Trochanter BMD	Control	0.601	(0.823)	0.598	(0.851)	-0.5	0.35	0.55
	Treatment	0.617	(0.112)	0.615	(0.110)	-0.32	0.33	
Hip BMD	Control	0.851	(0.114)	0.848	(0.116)	-0.35	0.69	0.3
	Treatment	0.889	(0.160)	0.895	(0.160)	0.67	0.48	
Total fat mass	Control	16.10	(6.75)	15.66	(6.57)	-2.71	0.06	0.22
	Treatment	18.28	(5.31)	17.98	(5.20)	-1.61	0.79	
Lean mass	Control	37.39	(7.36)	37.29	(7.16)	-0.26	0.52	0.2
	Treatment	40.25	(8.54)	40.35	(8.65)	0.23	0.54	

BMD (bone mineral density), BMC (bone mineral content

Conclusions

The improvement of total body BMC, total body BMD, BMDs of spine, femoral neck and hip were statistically significant among vitamin D treated patients compared to patients in the control group. Six months after stopping treatment the improvement in the regional BMD remained unchanged while only a marginal loss was observed in total body BMD and BMC.

Discussion

Most striking outcome of this randomized double-blind placebo controlled clinical trial conducted among patients with diabetic nephropathy was the significant reduction of urine microalbumin after monthly injection of vitamin D for six months. In addition, there was a significant reduction of serum creatinine and improvement of GFR among patients who received vitamin D. These results are supportive of the reno-protective effects of high dose vitamin D in diabetic patients with nephropathy who are on optimum medical therapy.

Further, vitamin D increased BMD / BMC compared to placebo given to patients in the control group. This improvement was observed in the total body BMD/BMC and BMDs of total hip, total spine and femoral neck. The regional BMDs remained unchanged six months after withdrawing vitamin D treatment while only a marginal loss was observed in total BMD and BMC.

Vitamin D caused no significant effect on cardiovascular risk scores, blood pressure or major serum lipid components except HDL.

In our sample we recruited patients in the early stages of renal disease (eGFR > 30 mL/min) and majority of them were not vitamin D deficient. Therefore we were able to increase their vitamin D levels to above physiological limits in order to examine for the non-classic benefits of vitamin D. These benefits were independent of the conventional treatment offered for these patients according to current treatment guidelines. According to the studies discussed above there was a significant reduction of urine microalbumin, serum creatinine and improvement of GFR after monthly injection of vitamin D for six months. These results are supportive of the reno-protective effects of high dose vitamin D in diabetic patients with nephropathy who are on optimum medical therapy. Furthermore, we observed a significant reduction of renin levels in the treatment group compared to the control group.

The dose of vitamin D used in this study raised serum vitamin D level substantially. Although this was sufficient to demonstrate reno-protective effect and benefit on BMDs the period of trail was insufficient to demonstrate a positive effect on CV measurements except HDL.

Based on the results of these studies vitamin D can be considered as an add-on therapy to patients with increasing microalbuminuria despite optimum glycaemic and blood pressure control and receiving maximum tolerable doses of ACEI or ARB.

Due to the paucity of data, however, further clinical trials should be done to reproduce the results observed in this study. If the same benefits are proven, use of vitamin D for complete suppression of albuminuria can be recommended.

Conclusions

Monthly injections of high dose vitamin D_3 has improved the renal functions, BMD and BMC in patients with diabetic nephropathy.

This treatment did not have a significant effect on cardiovascular risk scores or blood pressure except HDL levels.

Further studies involving longer durations of treatment at different doses of vitamin D may be needed to reconfirm these findings.

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Tissue micro array; establishing a cost-effective tool for cancer biomarker research in Sri Lanka

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ABSTRACT

Tissue micro array is a cost effective tool for cancer biomarker discovery and for the validation and external quality control in immunohistochemistry. It has not been utilized in Sri Lanka before, although widely used in cancer research centres world over. Scarcity of cancer biomarker research in Sri Lanka is partly due to the unaffordable cost of laboratory consumables including antibodies. TMA is produced using tissue cores from multiple tissue blocks. It reduces the cost and improves the consistency in immunostaining and adds validity to the assessment. In this brief report, we describe the technique of producing TMA and technical issues faced and how we could overcome them.

Key words: *TMA, cancer biomarkers*

Review

The concept of embedding tissue of different samples into one tissue block goes back to 1986 when the 'sausage' tissue blocks were developed for immunohistochemical assessment (1). The advantage of sausage block was that all of the tissue samples are treated equally during immunostaining and most sources of variation are eliminated which facilitates comparative studies. It was recommended for large scale inter-laboratory quality control processes. This concept was further developed and Tissue Micro Array (TMA) was designed to its current format by Kononen *et al* in 1998 (2). Now it is an invaluable research tool in cancer biomarker discovery.

TMAs are paraffin wax blocks (recipient blocks) constructed with tissue cores extracted from multiple tissue wax blocks (donor blocks). TMAs are sectioned and histology slides are prepared and can be stained with any routine histological stains and immunohistochemistry. It is a high-throughput technology useful in histology based laboratory tests and can be used in florescent in situ hybridization as well (3). TMA can also be used to assess molecular parameters (DNA, RNA) by molecular techniques. While TMA is made, a template per block is prepared indicating the reference number to map the clinical details of the patient with the biomarker score. Once made, TMAs can be used for subsequent assessment of multiple markers. Therefore, TMAs can be used as tissue libraries for future research. TMA cuts down on the cost for antibodies and reagents by many folds as a small core of representative tissue is carefully selected instead of a routine tissue sample. The selected size of the core can be 0.6 to 2 mm. Therefore, a TMA block can be built with hundreds of tissue cores minimizing the variation that can occur during staining procedures improving the validity and increasing the cost-effectiveness. In this brief report, we intend to describe our experience in how this technique can be established in a routine histopathology laboratory.

TMA blocks can be constructed manually or by using precision instruments. Automated forms of tissue micro arrayers are also available but less cost effective for a country like ours. In our histopathology laboratory we used a TMA Builder (Thermo Fisher™) and manually constructed TMAs for a research project on immunohistochemical biomarker assay for a cohort of breast cancer patients who's clinic-pathological and survival details were available for mapping and subsequent analysis.

Making a TMA block

The TMA Builder consists of a mould and a punch extractor. The base of the mould has 24 pins which makes 24 pits in the recipient block. The mould-top has an inset for C-ring and two lifting screws (Figure 1).

Paraffin wax pellets were melted at 60 °C in an oven to bring to liquid state. The base of the TMA mould was placed on a flat surface and a plastic C-ring was fitted into the inset in the mould-top. Molten wax was poured slowly to fill the C-ring which was then left to solidify. Once wax was solid enough, the C-ring filled with wax was removed from the metal mould by screwing down the two lifting-screws. The prepared wax block now has 24 pits to receive 24 tissue cores.

The donor tissue blocks were first examined for its physical suitability. The haematoxylin and eosin (H&E) stained slides of each case were reviewed. The best representative tumour region with minimum fixation artifacts was selected and marked for tissue extraction. The slide was superimposed on the corresponding donor block to identify the area in the tissue to be punched.

From each of these donor blocks, a core of 2 mm diameter tissue was extracted using the punch extractor of the TMA Builder™ (Thermo Fisher). The cores were transposed/injected into the pits in the recipient TMA wax mould prepared previously.

A core of brain tissue from a wax block was transposed into the 24th pit in the mould as a guide to identify the rows and the columns of the TMA. A template for each TMA block was prepared to link the biomarker score to clinico-pathological data of each case. We made 53 such TMA blocks containing breast cancer tissue of 1200 patients. Since the diameter of each tissue core was 2 mm which covers a sufficient surface area, tissue cores were not taken in duplicate (4).

TMA block was labeled in accordance with the template and was kept in the oven with the wax surface with tissue cores facing down on a flat metal surface. Temperature was set to 58 °C and left for 15 minutes to anneal the block and to bring the tissue cores to the cutting surface. Sections were cut at 4µ on a traditional microtome. Slides were kept overnight in the incubator at 60 °C before staining was done. Sections were assessed by light microscopy. TMAs also can be digitally scanned and displayed on a high resolution monitor (4). Scoring of the biomarkers on TMA was done blinded to the clinico-pathological data reducing the potential for bias. We were able to link this data to survival outcome and were able to prove the prognostic significance of immunohistochemical assessment of biomarkers in breast cancer which included new biomarkers (5). There are no other published reports on TMA technique being used for cancer research in Sri Lanka.

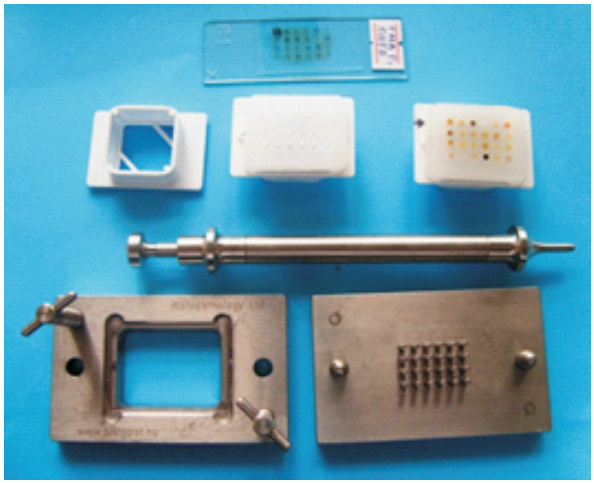

Figure1: This shows (items from top to bottom and from right to left) 1. A TMA slide stained for EGFR antigen; black circle indicating the guide core, 2.C-ring, 3. A recipient TMA paraffin block with 24 pits built on a C-ring, 4. A TMA block containing 24 tissue cores, 5. Punch extractor, 6. Mould-top of the TMA Builder with inset for C-ring and two lifting-screws in place, 7. Base of the TMA Builder with 24 pins.

Problems identified / troubleshooting

1. Locating the correct area to extract in a core biopsy donor block was difficult. The shape of the core of tissue which appeared on the block and matching it with the corresponding slide was used as a guide.
2. Since it is important to leave some diagnostic material in the block as archive, many core biopsies had to be excluded from our study. This was a limiting factor in preparing TMAs from core biopsies.
3. The depth to which the punch should cut into the donor block has to be first determined by trial as the extracted cores should be of the same length to fit into the pits in the recipient blocks.
4. Some tissue blocks were already sectioned extensively for the diagnostic process leaving only a thin piece of tissue and wax. The cores obtained from such blocks were very short compared to the depth of the TMA pit. The problem of shorter cores not reaching the cutting surface of the recipient block was resolved by keeping the TMA blocks in an oven as described in the annealing process. However, tissue loss was observed as such cores wore off after a few sections were obtained.
5. When the tissue in the recipient block contains fat around the tumour, correct superimposing of the slide to mark the correct site for core extraction was difficult. Inked resection margins, if available, were of help in such situations.
6. Breaking off of the outermost column of the tissue cores was frequently observed when the TMAs were sectioned. This occurred when the TMA blocks were fixed to the microtome through the plastic wings of the C-ring. The problem of breaking of blocks was overcome by fixing the TMA block through the frame of the C-ring.
7. Applying ice cubes on the surface of the TMA block just prior to sectioning, further reduced breakage of blocks.
8. Overnight incubation of tissue sections at 60 ^{o}C in the hot air oven, prior to immuno-histochemical staining, minimized the loss of tissue cores. This does not replace the necessity for a good section adhesive or a charged slide. Loss of tissue cores was minimal with H&E staining. When the guide core of the tissue section was lost during immunohistochemical staining, the H&E stained slide was very useful in identifying the location of the guide core. Therefore, it is advised to have the first section to be stained with H&E.
9. Folding of tissue sections was encountered at times. This occurred when the sections were too thick. Good microtomy skills were of utmost importance in obtaining sections of correct thickness without folds or cracks.

We believe that the information given in this brief report will be of value for Sri Lankan Histopathologists who wish to research into tumour biology and validate biomarkers using the plethora of cancer specimens they report and to find new knowledge on biomarkers at an affordable cost. It will be also useful in establishing external quality control system for immunohistochemistry laboratories in the country, which is a long-felt need.

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Compliance with Ethical Standards

This research project was granted approval from the Ethical Review Committee of the Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka.

Conflicts of interest

Author SNG received a monthly stipend as research assistant from the funding authority. The other authors declare that they have no conflicts of interest. The funding agency had no involvement in the study other than providing sufficient funds to conduct the research.

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Socioeconomic status and stress: Neuroendocrine pathways to disease

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ABSTRACT

The biomedical model investigates diseases of the organism at the cellular level, and ignores the broader socioeconomic factors that affect health. This discursive analysis examines the physiological mechanism by which socioeconomic factors get *inside* the human body and make people susceptible to diseases. A survey of recent materials was carried out using PubMed database to identify peer-reviewed manuscripts that examine physiological reaction to stress and its connection to diseases. These studies reveal a complex relationship between socioeconomic status and chronic diseases.

Key words: *Stress, adaptation, allostasis, allostatic load, illness*

Introduction

During the last three decades, researchers have been able to establish the physiological connection between socioeconomic status and diseases such as hypertension, coronary heart diseases, stroke, type II diabetes, memory impairment, and malignancies, which seem to increase as we go down the socioeconomic ladder of a society. While the social gradient may explain the continuous distribution of risks and vulnerabilities, the fundamental question that these researchers have been trying to answer was how does socioeconomic status get *inside* our bodies and make us susceptible to diseases? This discursive analysis, based on the current literature, explains the physiological mechanism through which socioeconomic status manifests as a major cause of disease, and makes some policy recommendations to mitigate the socioeconomic challenges, and their health damaging consequences.

Understanding the Social Determinants of Health:

The social determinants of health include a wide range of interrelated socioeconomic factors that affect people's everyday living. For example, income and employment status, the level of education, early childhood experience, and access to healthcare are some of the important social and economic factors that affect health throughout life. Yet, almost universally, these factors are not adequately incorporated into health policies and practices. Health has long been regarded as an outcome of good medicine. Although modern medical breakthroughs have eradicated many infectious diseases, the level of *exposure* to disease risks is often dependent upon a wide range of socioeconomic factors. It is only recently that policy makers have begun to examine population health within the broader context of socioeconomic status, and to introduce population health promotion strategies as part of the overall socioeconomic policies.

The social and economic problems influence health in every stage of life. For example, recent studies have shown that low birth weight and emotional deprivation in childhood are major causes of learning disabilities and other behavioral problems, which could in turn be a precursor to long-term material disadvantages in adult life. Moreover, these

material disadvantages are both absolute and relative: for example, not having money to buy food, or a place to live is a measure of an absolute poverty, which is a common problem among the people of the lowest rung of the social hierarchy. The effect of absolute poverty during in-utero growth through early childhood is known to produce biological markers for many adulthood diseases, such as diabetes and coronary artery diseases (1).

Relative poverty, on the other hand, is the difference between the standard of living enjoyed by the people in upper social echelons and those in the lower social classes in the same society. Relative poverty is believed to affect health through psychological and other status symbols. Although absolute poverty has significantly declined in recent years, relative poverty is rising throughout the world due to widening income gap between the rich and the poor. Studies have shown that in societies where socioeconomic disparities are higher, the overall mortality rate, infant mortality, violent crimes, hostility, obesity, and interpersonal distrust are greater (2). Thus, health is powerfully affected by both absolute and relative socioeconomic status. As we go down the social ladder within a given society, morbidity and mortality rates increase because the same people suffer from both types of deprivation. How does socioeconomic status get "under the skin," and make people sick?

Adaptation to Socioeconomic Pressure:

The link between socioeconomic status and disease is "stress," the term that often used to express the physical and psychological "pressure" exerted by various external challenges. These external challenges, which include socioeconomic problems, are powerful stressors in life. However, the impact of these stressors varies depending on the length of exposure to such challenges, the availability of supportive resources, and the individual genetic factors. Bruce McEwen defines stress as "a threat, real or implied, to the psychological or physiological integrity of an individual" (3). He recognizes, however, the ambiguity of the term as it is used in everyday life, which makes it difficult for us to understand how the human body copes with stress. As researchers have pointed out in recent years, this coping mechanism, which is described as *allostasis,* enables the organism to adapt to the external challenges, so that it can maintain the internal functional balance (*homeostasis*) of organ systems, and sustain life. Any physical or psychological challenge disturbs the functional balance of organ systems, and the body reestablishes it by adjusting neuroendocrine adaptation. Humans have survived on the earth by adapting to their physical and social environments. Overtime, however, the coping mechanism increases the vulnerability to major diseases, which is described as the *allostatic load*, or the cumulative wear and tear on the organ systems and their tissues due to prolong, insufficient, or failed adaptation (4).

As the brain determines the nature of the challenge, it regulates neuroendocrine response. Coping with any acute challenge requires extra energy for physical and psychological endurance. Thus, the autonomic nervous system, via the hypothalamic-pituitary-adrenal (HPA) axis, triggers the *catabolic* function of the digestive system resulting in an increase release of energy by breaking down protein, carbohydrate, and fat storages in the muscles and the liver. The release of three major endocrines (hormones), such as *epinephrine*, *norepinephrine,* and *dopamine* (collectively known as *catecholamine*) from the adrenal medulla increases the supply of blood to the heart, brain, and skeletal muscles. This in turn triggers fundamental physiological reactions affecting blood pressure: 1) the release of *epinephrine* triggers the discharge of the *glucagon* hormone by the pancreas to breakdown *glycogen,* the energy storages in the liver, which increases the plasma levels of glucose, free fatty acids, low-density lipoprotein, and cholesterol to provide more energy; 2) the discharge of *norepinephrine* stimulates the heart muscles to increase cardiac output (the heartbeat), and vasoconstrictions (tightening of the arteries) to eject more blood to the brain and skeletal muscles, and less blood to the gut and kidney; 3) *norepinephrine* stimulates the release of *renin-aldosterone* hormone in the kidney, which increases the plasma sodium concentration and arterial blood pressure. The *renin-aldosterone* mechanism increases the retention of sodium fluids in the blood flow, and prevents releasing the liquid through urine; 4) *dopamine*, primarily a neurotransmitter from the brain and autonomic nervous system, regulates the blood flow through the arteries, and the secretion of both *epinephrine* and *norepinephrine.* With the rising cardiac output, fluid

volume, and vasoconstriction, the blood pressure rapidly elevates forcing more blood to the brain and skeletal muscles, while restricting the blood flow to elsewhere in the body (5).

As these neuroendocrine mechanisms intensify the functional output of several organ systems, the release of the *glucocorticoid* hormone from the adrenal cortex regulates the *catabolic* process that increases the blood level of glucose. Obviously, all these physiological and neuroendocrine reactions to an external challenge require a fundamental shift in the performance of a number of major organ systems from an *anabolic* state of repairing tissues, producing immune cells, stimulating reproductive hormones, and storing excess energy to a *catabolic* state in which more energy is provided to certain organ systems to intensify their activity, while suppressing the performance of others temporarily in order to sustain life (or to face the challenge).

Adaptation and Disease:

The stress induced disease, or *allostatic load,* is the chronic wear and tear on the body resulting from adaptation to stressor. As noted, a common outcome of the adaptation (*allostasis*) is cardiovascular reactivity resulting in high blood pressure. The elevated blood pressure accompanies several risk factors such as increased blood levels of glucose, low-density lipoprotein, and cholesterol, which are all known clinical risk factors for heart diseases, diabetes, obesity, and strokes. Studies have shown that once the neuroendocrine process raises the blood pressure, and maintains it at a higher level for an extended period, blood pressure tends to stay high even if the initial cause of the elevation no longer exists. The brain and arteries develop structural and functional remodelling as part of the adaptation. This remodelling, over time, particularly in connection with arterial constriction, causes damage in the inner lumen of the arterial walls, where atherosclerotic plaques begin to develop, obstructing the blood flow. At this stage, even if the fluid volume has become normal, the narrowed arteries with blockages are bound to cause heart attacks and strokes (6).

Also, the release of *glucocorticoid* hormone during stress undermines the production of white blood cells, which has several pathological consequences. During prolonged stress the suppressed immune system delays the healing of wounds and injuries, and most importantly exposes the organism to various pathogenic agents such as viruses, bacteria, and carcinogenic agents, which are normally removed from the body by white blood cells. The immune system, particularly the thymus gland and its ability to produce white blood cells, is mediated by *glucocorticoids* (7).

Likewise, under chronic stress, *glucocorticoid* impedes the insulin function that regulates the blood glucose level. The function of insulin is to remove the extra glucose from the blood and store them in the muscles, body fat, and the liver as *glycogen* to be utilized when the blood glucose level goes down. During stress, the increased demand for energy to meet an external challenge prevents this insulin activity resulting in abnormally high levels of blood glucose. While elevating blood levels of glucose and free fatty acids, and preventing insulin from storing them in the muscles and the liver, the *glucocorticoid* promotes the deposition of *glycogen* in the abdomen. It has been identified that abdominal obesity, as in Cushing's disease, as well as type I and II diabetes are triggered by chronic stress (8). A number of recent studies have substantiated these observations reporting that excessive metabolic disorders among low-income groups, such as African-Americans, are related to psychosocial stress of living in hierarchical societies (9). Kate Pickett and colleagues found that in the top 50 developed countries, adjusting for gross national per capita income, the distribution of income is *positively* correlated with a number of health indicators such as the percentage of obese people in the population, diabetes-related mortality rate, and the average daily per capita calories intake (10).

While the brain is the main organ that regulates the neuroendocrine response to stress, it is also subjected to major neurological remodeling and structural transformations due to prolonged stress. Both animal and human studies have shown that prolonged activation of *glucocorticoid* receptors of the brain increases the risk of protein plaques deposition on the neurons obstructing the chemical messengers (neurotransmitters). The increased blood flow to the brain also carries with them high levels of plasma protein that accumulates on the neurons over time, eventually destroying them altogether. The *amyloid hypothesis* suggests that the accumulation of *amyloid* plaques on neurons may be linked to Alzheimer's disease. Recent studies of type II

diabetes, which is connected to increased plasma level of *glucocorticoid* under chronic stress, found reduced hippocampal volume (shrinking of the hippocampus). They have also shown that the reduced hippocampal volume is associated with memory impairment and cognitive dysfunction, and a general relationship between the size of the hippocampus and spatial memory (11). *Studies based on* human neuroimaging have shown that prolonged stress and post-traumatic stress disorders produce drastic structural changes in the prefrontal cortex, hippocampus, and amygdala leading to chronic depressive symptoms and anxiety disorders (12).

The neuroendocrine reactions triggered by socioeconomic challenges inevitably impose undue pressure on the organ systems and their tissues in the long-term. Generally, it is constructive when the neuroendocrine reactivity is rapidly mobilized on the organ systems and terminated immediately. However, when the neuroendocrine reactivity is prolonged due to chronic stress, it undermines mental and physical health. How rapidly those neuroendocrine mechanisms are activated and are halted is dependent upon the availability of effective coping mechanisms and resources for individuals, families, and communities. Unhealthy coping mechanisms could further aggravate damaging health consequences.

Behavioural Adaptations as Coping Mechanisms:

The pathophysiological conditions resulting from the adaptation to stress are often exacerbated by a variety of harmful behavioural adaptations, such as smoking, drinking, overindulging, and the lack of physical activity, which are often perceived as coping strategies by some individuals. These behavioural adaptations have the same pathogenic outcomes for major organ systems, as do the physiological adaptions to stress.

By contrast, however, studies have shown that the availability of social support, trusting interpersonal relations, regular physical activity, and membership in community organizations, which have been collectively described as *social capital*, enhances the resilience of organ systems. Any supportive relationship that has an attenuating effect on psychophysiological stress may have a positive long-term impact on health (13). People with social networks receive both material and emotional support during stressful situations in their lives. Social connectivity and supportive environments are known to increase inhibitory signals to the HPA axis, which in turn raises the HPA activation threshold thereby minimizing the effect of potential socioeconomic challenges to physiological system. In other words, if the individual is confident that support is available at a time of "distress" such knowledge could prevent the full-blown stress response from the HPA axis. The individual differences in social integration and connectivity (social networks) could therefore modify the cognitive perceptions of external challenges, which may in turn moderate the neuroendocrine reactions to such challenges (14).

Conclusion: Policy Recommendations

In recent years, particularly in developed Western countries, public policies on population health promotion have increasingly been incorporated into major economic policies. There has been significant effort to reduce the social gradient in health through inclusive economic policies, which have the potential to increase prosperity, greater redistribution of resources, and the overall social cohesiveness. These policies are results of several well-recognized public inquiries into the impact of socioeconomic inequalities in some countries, and the global campaigns by various multilateral organizations, such as the World Health Organization (WHO) and the World Bank, to inform about the "health cost" of inequality and economic marginalization. For example, the report of the Donald Acheson Commission of the United Kingdom, and the WHO Commission on Social Determinants of Health advocate that population health strategies to be based on four major pillars to counter the potential impact of material disadvantages from early childhood through adulthood: 1) reducing poverty and income inequality through targeted redistributive welfare policies to give greater educational and employment opportunities for disadvantaged social groups; 2) preventing unhealthy life-styles and life course issues, such as smoking, drinking, and malnutrition to promote healthy development; 3) prohibiting discrimination based on age, gender and ethnicity in areas such as employment, education and housing to

ensure social justice and equality; 4) ensuring universal access to quality healthcare, particularly primary care (15).

The underlying philosophy that guides these broad policy recommendations is that good health for all can be achieved by providing a good "foundation" in life through guaranteed access to education, healthcare and employment. Life is a progression through stages, in that material disadvantages in early childhood that prevent getting a good education, undermines the labour market opportunities during adulthood. People who have experienced disadvantages in early childhood are at the greatest risk in the subsequent stages of life. Policies need to prevent people from experiencing disadvantages at the earliest possible stages of life, and they need to give priority to those who are already at a disadvantage in society. These are the people who suffer from both absolute and relative deprivations.

The social environments–at work, school, or neighbourhood–that provide a sense of belongingness, security and safety are known as "healthy environments" as they prevent marginalization and deprivation, and promote a sense of well-being. In such social environments, individuals are less likely to make unhealthy lifestyle choices. By contrast, environments, in which people feel excluded, abused and discriminated, are regarded as "toxic environments," where people feel less worthy of themselves, seek the comfort of health damaging behaviours, and engage in criminal activities. Therefore, national and local authorities, as well as public and private employers, and community leaders need to recognize the health implication of social integration in institutions, as well as, in communities and neighbourhoods. Policies that prevent discrimination, abuse and violence not only increase economic productivity, but also promote health and well-being. People who are more socially connected: 1) live longer; 2) are more likely to survive a myocardial infarction; 3) are less likely to experience a recurrence of cancer; 4) are less likely to suffer from infectious illness, than those who are less integrated to the community (16).

Likewise, access to healthcare is a basic human right that has been recognized by the World Health Organization. Specifically, the access to primary care is critical for preventing disease, immunizing children and providing prenatal care services that build the foundation for healthy living. Although health care services themselves focus on the clinical aspects of risks rather than the social determinants of exposure to risks, preventive health services protect people from diseases and premature death. Public policies must ensure that all citizens have guaranteed access to essential health services, so that an unforeseen illness would not prevent people from fully participating in education and employment resulting in life-long socioeconomic disadvantage. Public policies need to address the socioeconomic determinants of health before they manifest as major health problems. This is the challenge for both policy makers and political leaders.

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Exploring the pleiotropic effects of vitamin D in diabetes

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Introduction

Vitamin D is a unique vitamin and a hormone which is absolutely essential for bone mineral homeostasis. In addition, in the recent past there has been a growing appreciation for its role as an endocrine regulator having important roles in the renal, cardiovascular, reproductive and immune systems in the body. These actions are named non-classic actions or pleiotropic effects of vitamin D.

Regarding the non-classic actions of the hormonal form of vitamin Din the renal system, inhibition of Renin Angiotensin System (RAS)by reducing renin synthesis is an important action of vitamin D . Renin is the first and the rate limiting step of the RAS cascade.

This action of vitamin D can be used to overcome the unwanted effects resulting from the long term use of Angiotensin Converting Enzyme Inhibitors (ACEI) and Angiotensin Receptor Blockers (ARB) in patients with renal and cardiovascular disease; that is compensatory rise in renin level as a result of disruption of feedback inhibition of renin production.

ACEI and ARB are included in the standard treatment for reducing proteinuria in diabetic nephropathy, which is the commonest cause of end stage renal disease worldwide . Despite the treatment with ACEI and ARB, rate of progression of diabetic nephropathy remains high. Compensatory rise in plasma renin level leading to activation of RAS is one important mechanism which has been postulated as a possible reason for the inadequacy of ACEI and ARBs on reversing diabetic nephropathy.

Current data on the role of vitamin D reducing the progression of renal damage in diabetic renal disease are limited and come from either animal studies or few human studies . Studies which explored the effect of vitamin D on animal models of kidney disease have reported the beneficial effects of such therapy. Randomized control trials examining the effect of vitamin D on the progression of proteinuria are limited ,. Further, these studies are not sufficiently powered to generate conclusive results. There is a paucity of sufficiently powered randomized controlled trials examining the different reno-protective effects of vitamin D among patients with diabetic nephropathy.

Hence we planned to evaluate the effect of vitamin D therapy in reversing the progression of diabetic nephropathy and also to determine the effect on plasma renin, cardiovascular disease risk (CVDR) profile, bone mineral density (BMD) and bone mineral content (BMC) measurements.

This review includes the following arms exploring the effects of vitamin D on

1. renal functions in patients with diabetic nephropathy.
2. lipid profile and blood pressure in patients with diabetic nephropathy.
3. cardiovascular risk scores in patients with diabetic nephropathy.
4. bone mineral density in patients with diabetic nephropathy.

Methods

This study was conducted on patients with diabetic nephropathy (urinary albumin >30 mg/g of creatinine in two occasions) whose GFR was more

than 30 mL/min. Patients who had albuminuria in a previous cross sectional study six months back were invited and investigations were repeated. This procedure ensured confirmation of albuminuria at least on two occasions over a period 6 months. Selected patients were informed about the study and written consent was obtained. Those who had blood pressure > 130/80 mmHg during the last two clinic visits, hyperphosphataemia (serum phosphate > 5 mg/dL), hyper or hypocalcaemia, uncontrolled blood sugar (current HbA1c > 8) and those with liver disease, hyperthyroidism, hyperparathyroidism, or diseases related to calcium or vitamin D metabolism and congestive heart failure (current) were excluded. Attempts were made to exclude other causes of proteinuria such as ongoing urinary tract infection, urolithiasis, and renal tuberculosis by history, examination and previous investigations. Morning urine samples were collected and urine dipstick test was done to exclude ongoing urine infections. Urine collection was postponed if a patient had fever, urinary symptoms, or menstruation. Only those were negative for nitrates were stored in -80^{0}C for urine albumin analysis. After two weeks second sample of urine was collected. Same procedure was followed as for the first urine sample. If the urinary albumin excretion of the second sample was inconsistent with the first sample, a third urine sample was checked. Presence of microalbuminuria was confirmed only if two consecutive or two out of three samples were positive for urine albumin > 30 mg albumin/g of creatinine. In the same manner, macroalbuminuria was confirmed when urine albumin excretion was > 300 mg/g of creatinine.

Study design

Patients were allocated to two groups by block randomization method (block of 2) using a random number table. Concealed envelopes containing treatment allocation were given to research assistants who assigned participants to treatment and control groups. Treatment group received monthly dose of 50,000 IU of vitamin D_3 intramuscularly and the control group was given an equal volume of distilled water (0.25 mL) to the same site in a similar manner. Participants, those administering the interventions, clinicians, and those assessing the outcomes were blinded to the group assignment.

Study Procedures

Patients underwent a detailed medical history, a physical examination including systolic and diastolic blood pressure (SBP and DBP) measurement. Blood and urine were collected for the baseline measurements which included serum creatinine, serum calcium, urine microalbumin, fasting blood glucose (FBS), serum calcium, phosphate, creatinine, Parathyroid Hormone (PTH), renin and vitamin D level and lipids namely total cholesterol (TC), low density lipoprotein (LDL), triglycerides (TG), and high density lipoprotein (HDL). CVDR was calculated using Framingham risk score (FRS). The CVDR, fatal coronary vascular disease risk (FCVDR), stroke risk (SR) and fatal stroke risk (FSR) were obtained by the UKPDS (United Kingdom Prospective Diabetes Study) risk engine.

A safety visit was scheduled 1 week after starting the treatment to monitor the serum Ca and phosphorus concentrations and to elicit any adverse events. The protocol permitted withdrawal from the trial if serum Ca exceeded 11 mg/dL. During monthly visits patients were inquired about side effects according to a check list in the data collection sheet.

All patients underwent whole body dual-energy X-ray absorptiometry (DXA) scan and BMD and BMC of the total body, total spine (L_1-L_4) and proximal femur were measured. All scans were performed and analysed by the same technician adhering to the manufacturer's protocol. DXA machine was calibrated using the calibration phantom provided by the manufacturer. The precision error of the machine has been published previously . There were no software or hardware changes during the study period.

At three months blood samples were collected for serum creatinine, serum calcium, serum phosphate, FBS, lipid profile and urine was taken for the assessment of urine micro albumin to creatinine ratio at three months.

After six months of treatment all the measurements done at the baseline, including DXA were repeated. When the trial period of six months was over, a randomly selected subgroup of patients (25 from each group) was followed up for further six months and another DXA testing was performed.

Biochemical assays were performed using commercial kits. Urine albumin was measured by turbid metric method while urinary creatinine concentration was measured using an end-point spectrophotometric method with an alkaline-picrate solution.

Serum creatinine was measured by auto-creatinine calibrated with autocal which is traceable to reference material. Glomerular Filtration Rate (GFR) was estimated by CKD-EPI equation.

Intact PTH (Immunotech, IRMA PTH), renin (Beckman coulter, IRMA Active Renin) by radioimmunoassay and 25-hydroxy vitamin D were measured using immunochemiluminometric (Vitros immunodiagnostic) assays. Serum creatinine was measured by spectrophotometric method with an alkaline-picrate solution.

Ethical aspect

Ethical clearance was obtained from the Ethics Review Committee of the Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka.

Clinical trial has been registered in the local clinical trial registry. Informed written consent was obtained from all subjects of the study.

Statistical analysis

The baseline characteristics between the two groups were compared by either unpaired t-test or Chi-square test. Changes in urinary albumin, renal functions, vitamin D, renin, PTH, BMD/BMC during the trial period were analyzed by the Repeated measure ANOVA (SPSS, Chicago, USA). P value was adjusted for multiple comparisons by the Bonferroni method.

Results

A total of 157 patients were invited for the study and 72 were excluded due to the presence of one or more exclusion criteria. Remaining 85 were randomly assigned to two groups and 82 subjects completed the study; 41 patients from each group completed the study.

No significant differences were found with regards to the baseline characteristics between the treatment and control groups (Table 1).

Table 1: Baseline characteristics of subjects in the two groups

Variable	Control group (n = 43)	Treatment group(n = 42)	*P* value
Age (years)	59 (8)	56 (10)	0.1
Number of males (%)	42.9	48.8	0.58
SBP (mmHg)	121 (7)	120 (8)	0.46
DBP (mmHg)	70 (5.9)	71 (5.9)	0.25
HbA_1C (%)	7.1 (0.5)	6.9 (0.5)	0.1
Calcium (mg/dL)	8.9 (0.7)	8.8 (0.6)	0.65
Phosphorus (mg/dL)	3.8 (0.6)	3.9 (0.5)	0.31
PTH (pg/mL)	42.5 (19.0)	38.2 (11.3)	0.21
Plasma renin (pg/mL)	15.14 (4.82)	14.64 (5.62)	0.66
25(OH)D (nmol/L)	49.6 (16.5)	56.1 (12.9)	0.07
FBS (mg/dL)	130 (12.5)	128 (13.3)	0.51
Duration of diabetes (years)	7 (4)	8 (5)	0.42
Urine creatinine (mg/dL)	63.6 (10.9)	61.7 (11.9)	0.44
Urine albumin (mg/g of creatinine)	185.8 (50.6)	164.4 (35.8)	0.83
Glomerular Filtration Rate (mL/min)	83.2 (16.1)	86.7 (14.6)	0.28
HDL (mg/dL)	53.5 (10.9)	50.3 (7.5)	0.13
TC (mg/dL)	194.6 (32.1)	194.8 (30.1)	0.87
LDL (mg/dL)	117.0 (28.1)	119.7 (28.7)	0.87
TG (mg/dL)	128.4 (50.8)	122.8 (41.4)	0.66
BMI (kg/m^2)	23.2 (4.0)	24.4 (3.4)	0.14

SBP (systolic blood pressure), DBP (diastolic blood pressure), PTH (parathyroid hormone), FBS (fasting blood sugar), HDL (high density lipoprotein), TC (total cholesterol), LDL (low density lipoprotein), TG (triglyceride), BMI (body mass index)

All patients received either an ARB or ACEI at the baseline. During the study period, oral hypoglycemic drugs were increased in nine patients (6 in the treatment group). Losartan was increased in three patients (2 in the control group). Blood pressure number and doses of anti-hypertensive drugs were quite stable during the clinical trial.

1. Vitamin D therapy on renal functions of patients with diabetic nephropathy

Diabetic nephropathy is the leading cause of end stage renal disease and despite optimum therapy including ACEI/ARBs, a sizable proportion of patients with proteinuria progress to renal failure. It is likely that high renin level induced by RAS (Renin Angiotensin System) blockage may contribute to this and vitamin D is found to have an inhibitory effect over RAS as it reduces renin synthesis.

This study was conducted to examine the effects of vitamin D therapy on renal functions of patients with diabetic nephropathy.

Effect of vitamin D therapy on urinary albumin excretion, renal functions and plasma renin among patients with diabetic nephropathy; a randomized, double-blind clinical trial.

Results

Table 2 shows the changes of the urine microalbumin to creatinine ratio, systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting blood sugar (FBS), serum creatinine, eGFR, PTH, renin and vitamin D levels after three and six months of treatment in the treatment and control groups. After six months, mean reduction of urinary albumin to creatinine ratio was 51.8 mg/g ($P = < 0.001$) in the treatment group, 22.4 mg/g ($P = 0.06$) in the control group and this difference was significant ($P = 0.001$). Significant increase in the GFR was observed in the treatment group while in the control group GFR remained unchanged ($P = 0.03$ for the between-groups difference). There was a significant reduction of serum creatinine in the treatment group but not in the control group. But the change was not significant between groups.

A significant increase of SBP was seen in the control group whereas SBP remained unchanged in the treatment group and the difference was not statistically significant. Significant trends in the DBP was seen in both groups during the study period but the difference between the two groups was not statistically significant ($P = 0.17$). Significant reduction of FBS was seen only in the control group and the difference between groups was not statistically significant ($P = 0.23$).

Significant reduction of PTH was observed in both treatment and the control groups. But the change between two groups was not statistically significant ($P = 0.26$). In the treatment group, vitamin D level increased by 25.64 nmol/L and between the two groups the change was statistically significant ($P < 0.001$). Mean reduction in plasma renin in the treatment group was 5.85 pg/mL ($P < 0.001$). In the control group the reduction observed was only 0.95 pg/mL. The difference between the two groups was statistically significant ($P = 0.006$) (Table 2).

A significant inverse correlation was observed in vitamin D with percentage change in plasma renin level ($\rho = -0.66$, $P < 0.01$) and percentage change in urine albumin levels ($r = -0.47$, $P < 0.01$). Furthermore, percentage changes of renin and urinary albumin also showed a significant correlation ($\rho = 0.62$, $P < 0.01$) (Table 3).

According to the table 4, the microalbuminuria suppression is related to the final serum renin level. The suppression of microalbuminuria is highest in the highest tertile of serum renin and it does not vary across the tertiles of serum vitamin D. The lowest suppression of microalbuminuria is in the lowest tertile of the serum renin. Furthermore, there is a gradient of microalbuminuria suppression across the tertiles of the serum renin concentration.

Serum Ca in patients with vitamin D treated and in the control group were 9.16 (0.61) and 9.045 (0.69) at the end of trial. The difference was not statistically significant. No adverse events, particularly hypercalcaemia were reported during the study period.

Table 2: Changes observed in the treatment and control groups at 3 months and 6 months

Variable		Baseline	At 3 months	At 6 months	*P* within group	*P* between group
SBP (mmHg)	Control	121 (7)	121 (8)	127 (6)	< 0.001	0.07
	Treatment	120 (8)	120 (8)	121 (7)	0.59	
DBP (mmHg)	Control	70 (6)	72 (6)	72 (6)	< 0.001	0.17
	Treatment	71 (6)	69 (6)	68 (6)	< 0.001	
FBS (mg/dL)	Control	130.2 (12.5)	130.6 (10.1)	127.8 (10.7)	0.02	0.23
	Treatment	128.3 (13.6)	125.8 (13.4)	125.9 (10.9)	0.08	
PTH (pg/mL)	Control	42.5 (19.0)		37.6 (12.6)	0.003	0.26
	Treatment	38.2 (11.3)		35.7 (7.9)	0.001	
25 (OH)D (nmol/L)	Control	49.64 (16.46)		45.67 (17.20)	0.004	< 0.001
	Treatment	56.11 (12.95)		81.75 (15.03)	< 0.001	
Plasma renin (pg/mL)	Control	15.14 (4.82)		14.19 (4.6)	0.02	0.006
	Treatment	14.64 (5.62)		8.83 (4.81)	< 0.001	
Urine albumin (mg/g)	Control	185.8 (50.6)	160.9 (63.4)	163.4 (56.2)	0.06	0.001
	Treatment	169.4 (35.8)	122.1 (54.4)	117.6 (45.2)	< 0.001	
Serumcreatinine (mg/dL)	Control	0.87 (0.22)	0.87 (0.20)	0.87 (0.20)	0.84	0.10
	Treatment	0.86 (0.13)	0.80 (0.12)	0.77 (0.11)	< 0.001	
GFR (mL/min)	Control	83.2 (16.1)	83.4 (15.6)	83.9 (14.9)	0.74	0.03
	Treatment	86.7 (14.6)	90.7 (14.8)	93.7 (14.1)	< 0.001	

SBP (systolic blood pressure), DBP (diastolic blood pressure), PTH (parathyroid hormone), FBS (fasting blood sugar), GFR (glomerular filtration rate)

Table 3: Correlations (Spearman rho) between the percentage change in vitamin D, urine albumin, plasma renin and PTH

Percentage change	Urine albumin	Renin	PTH
Vitamin D	-0.47**	-0.66**	-0.02
Urine albumin		0.62**	-0.08
Renin			-0.02

**Correlations are significant at 0.01 level.

Table 4: Percentage change in urinary albumin excretion in relation to of vitamin D and renin

Vitamin D ↓	Renin → Low	Middle	High
Low	32.9 (18.3)	11.9 (7.4)	5.7 (9.1)
Middle	45.0 (8.1)	10.0 (11.6)	2.9 (8.6)
High	33.5 (6.9)	12.6 (11.6)	5.8 (8.6)

Values are given mean (SE)

Conclusions

Randomized double-blind placebo controlled clinical trial conducted among patients with diabetic nephropathy showed a significant reduction of urine microalbumin, serum creatinine, renin levels and improvement of GFR among patients in the treatment group compared to the control group after monthly injection of vitamin D for six months.

2. Vitamin D therapy on lipid profile and blood pressure in patients with diabetic nephropathy

Effects of six month, high-dose parenteral vitamin D therapy on lipid profile and blood pressure in patients with diabetic nephropathy; a randomized double-blind clinical trial

Aim of this study was to determine the effect of high dose vitamin D given to patients with early diabetic renal disease on systolic and diastolic blood pressure, total cholesterol (TC), low-density lipoproteins (LDL), triglycerides (TG) and high density lipoproteins (HDL) in a randomized controlled trial.

Results

Table 5 shows the changes in SBP, DBP, TC, TG, LDL, HDL and vitamin D at 3 and 6 months of follow up.

In the treatment group, vitamin D level increased by 25.64 nmol/L and between the two groups the change was statistically significant (P < 0.001).

Vitamin D therapy significantly reduced DBP, total cholesterol and LDLC in the treatment group, but the between group differences were not significant. There was an increase in HDL cholesterol level in the treatment group while there was no change in the control group (the between groups difference was significant).

Table 5: Changes in CVDR factors in the treatment and control groups

Variable		Baseline	At 3 months	After 6 months	*P* value within group	*P* value between group
SBP	Control	121 (7)	121 (8)	127 (6)	< 0.001	0.07
(mmHg)	Treatment	120 (8)	120 (8)	121 (7)	0.59	
DBP	Control	70 (6)	72 (6)	72 (6)	< 0.001	0.17
(mmHg)	Treatment	71 (6)	69 (6)	68 (6)	< 0.001	
TC	Control	194.6 (32.1)	193.6 (30.8)	196.9 (31.4)	0.24	0.50
(mg/dL)	Treatment	194.8 (30.1)	191.5 (28.1)	185.7 (27.2)	< 0.001	
TG	Control	128.4 (50.8)	127.9 (49.5)	128.7 (45.3)	0.62	0.44
(mg/dL)	Treatment	122.8 (41.4)	121.8 (40.1)	118.2 (32.4)	0.062	
LDL	Control	117.0 (28.1)	114.6 (28.9)	117.1 (30.2)	0.34	0.7
(mg/dL)	Treatment	119.7 (28.7)	115.7 (27.6)	106.10 (26.5)	< 0.001	
HDL	Control	35.5 (10.9)	53.7 (10.7)	53.9 (9.7)	0.40	< 0.001
(mg/dL)	Treatment	50.3 (7.5)	51.5 (7.1)	55.7 (6.8)	< 0.001	
25 (OH)D	Control	49.64 (16.46)		45.67 (17.20)	0.004	< 0.001
(nmol/L)	Treatment	56.11 (12.95)		81.75 (15.03)	< 0.001	

SBP (systolic blood pressure), DBP (diastolic blood pressure), TC (total cholesterol), TG (triglyceride), LDL (low density lipoprotein), HDL (high density lipoprotein)

Conclusions

Randomized double blind placebo control clinical trial conducted among patients with diabetic nephropathy has shown significant improvement in HDL levels but no significant effect on blood pressure after monthly injection of vitamin D for six months.

3. Vitamin D therapy on cardiovascular risk scores of patients with diabetic nephropathy

Effects of six month, high-dose parenteral vitamin D therapy cardiovascular risk scores in patients with diabetic nephropathy; a randomized double-blind clinical trial

Diabetes is considered as a coronary vascular disease equivalent. Cardiovascular disease risk (CVDR) can be assessed by cardiovascular risk scores. The aim of this study was carried out to assess the effects of vitamin D therapy on CVDR scores among patients with diabetic nephropathy.

CVDR was calculated using Framingham risk score (FRS). Further, CVDR, fatal coronary vascular disease risk (FCVDR), stroke risk (SR) and fatal stroke risk (FSR) were obtained by the UKPDS (United Kingdom Prospective Diabetes Study) risk engine.

Results

No significant differences were found between treatment and control groups at the baseline. FRS, CVDR, FCVDR, SR and FSR values before and after the intervention were 20.32 and 21.41, 13.62 and 11.94, 8.97 and 7.951, 7.66 and 7.50, 0.95 and 0.94 respectively in the treatment group. Respective values of the control group were 19.96 and 22.49, 12.76 and 13.59, 7.902 and 8.80, 6.11 and 6.66, 0.75 and 0.87.

No significant effect was found with vitamin D_3 treatment on CVDR scores, measured by FRS and UKPDS (Table 6).

Table 6: Changes in CVD risk scores in the treatment and control groups

Variable		Baseline	At 3 months	At 6 months	*P* value within	*P* value between
FRS	Control	19.96 (11.09)	19.79 (12.02)	22.49 (11.69)	< 0.001	0.92
	Treatment	20.32 (14.14)	19.69 (13.73)	21.41 (21.61)	0.62	
CVDR	Control	12.76 (08.33)	12.72 (08.37)	13.59 (08.69)	.004	0.88
	Treatment	13.62 (11.27)	12.57 (09.85)	11.94 (09.55)	< 0.001	
F CVDR	Control	7.902 (6.32)	8.05 (6.10)	8.80 (6.60)	0.006	0.96
	Treatment	8.97 (9.40)	8.09 (7.82)	7.951 (7.10)	0.005	
Stroke risk	Control	6.11 (4.35)	6.15 (4.40)	6.66 (4.70)	< 0.001	0.51
	Treatment	7.66 (9.60)	6.80 (7.70)	7.50 (9.00)	0.11	
F Stroke risk	Control	0.75 (0.55)	0.76 (0.56)	0.87 (0.62)	< 0.001	0.55

Framingham risk score (FRS), Cardiovascular disease risk (CVDR), fatal coronary vascular disease risk (FCVDR), fatal stroke risk (F Stroke risk)

Conclusions

Monthly injections of high dose vitamin D_3 but did not have a significant effect on cardiovascular risk scores among patients with diabetic nephropathy.

4. Vitamin D therapy on bone mineral density of patients with diabetic nephropathy

Effect of vitamin D therapy on bone mineral density among people with diabetic nephropathy; a randomized, double-blind placebo controlled clinical trial.

Aim was to determine the effect of vitamin D given to patients with diabetic nephropathy on bone mineral density (BMD) and bone mineral content (BMC).

Results

A total of 157 people were invited for the study and 72 were excluded due to the presence of one or more exclusion criteria. Remaining 85 were randomly assigned to two groups; 43 subjects in the treatment group and 42 subjects to the control group. No significant differences were found with regards to the baseline characteristics between the treatment and control groups.

Two participants from the treatment group and one participant from the control group did not complete the study. They were not contactable probably due to the change of the residence. Forty one subjects from each group completed the intervention. At the end of 6 months DXA results were available in 39 participants in the treatment group and 38 in the control group. At the end of one year, 25 from each group underwent the 3rd BMD measurement.

Table 7 shows the changes of the total body BMD/BMC, regional BMDs, total fat and lean masses, during the initial six months of treatment in the treatment and control groups.

After six months of vitamin D injections, total body BMD, total body BMC and BMDs of spine, femoral neck and total hip regions increased by 2.0%, 2.2%, 1.8%, 2.1% and 2.6% ($P < 0.05$ for all), respectively from the baseline figures. However, increase observed in the trochanteric BMD among them was not statistically significant. In the control group, compared to the baseline values, total body BMC, BMD, or regional BMDs did not change significantly during the initial six months.

Table 7: Changes bone mineral density and fat mass in the treatment and control groups

Variable		Baseline	After 6 months	Percentage difference	*P* within groups	*P* between groups
BMD	Control	1.038 (0.121)	1.031 (0.191)	-0.67	0.75	0.61
	Treatment	1.038 (0.120)	1.059 (0.107)	2.02	0.01	
BMC	Control	1775.63 (412.76)	1721.64 (369.70)	-3.04	0.074	0.73
	Treatment	1757.95 (383.68)	1795.85 (373.27)	2.16	0.007	
Spine BMD	Control	0.848 (0.132)	0.836 (0.119)	-1.41	0.27	0.72
	Treatment	0.845 (0.153)	0.860 (0.142)	1.78	0.04	
Femoral neck BMD	Control	0.722 (0.109)	0.712 (0.094)	-1.38	0.23	0.43
	Treatment	0.731 (0.153)	0.746 (0.142)	2.05	0.03	
Trochanter BMD	Control	0.607 (0.089)	0.604 (0.08)	-0.49	0.5	0.46
	Treatment	0.615 (0.111)	0.627 (0.103)	1.95	0.07	
Hip BMD	Control	0.857 (0.113)	0.852 (0.105)	-0.58	0.56	0.25
	Treatment	0.876 (0.148)	0.899 (0.149)	2.62	0.008	
Total fat mass	Control	15.85 (6.67)	16.48 (6.16)	3.99	0.20	0.2
	Treatment	17.41 (5367.460)	18.21 (5.56)	4.6	0.06	
lean mass	Control	37.04 (6.94)	36.98 (6.38)	-0.18	0.86	0.16
	Treatment	38.92 (8.32)	39.64 (7.73)	1.85	0.09	

BMD (bone mineral density), BMC (bone mineral content

Six months after the cessation of vitamin D treatment, significant reductions of both total BMD and BMC were observed (P = 0.009) while regional BMDs remained unchanged (Table 8). In the control group none of the BMD / BMC measurements changed significantly during the post-trial follow up six months period.

Table 8: Changes bone mineral density and fat mass in the treatment and control groups

Variable		After 6 months		After 12 months		Percentage difference	*P* within groups	*P* between groups
BMD	Control	0.999	(0.134)	1.006	(0.112)	0.70	0.47	0.21
	Treatment	1.054	(0.120)	1.041	(0.131)	-1.23	0.009	
BMC	Control	1735.92	(430.22)	1716.05	(402.87)	-1.14	0.26	0.54
	Treatment	1808.19	(450.57)	1795.94	(458.27)	-0.68	0.04	
Spine BMD	Control	0.823	(0.128)	0.828	(0.126)	0.61	0.19	0.69
	Treatment	0.847	(0.168)	0.837	(0.163)	-1.18	0.07	
Femoral neck BMD	Control	0.711	(0.109)	0.711	(0.105)	0	0.92	0.28
	Treatment	0.756	(0.166)	0.753	(0.169)	-0.4	0.48	
Trochanter BMD	Control	0.601	(0.823)	0.598	(0.851)	-0.5	0.35	0.55
	Treatment	0.617	(0.112)	0.615	(0.110)	-0.32	0.33	
Hip BMD	Control	0.851	(0.114)	0.848	(0.116)	-0.35	0.69	0.3
	Treatment	0.889	(0.160)	0.895	(0.160)	0.67	0.48	
Total fat mass	Control	16.10	(6.75)	15.66	(6.57)	-2.71	0.06	0.22
	Treatment	18.28	(5.31)	17.98	(5.20)	-1.61	0.79	
Lean mass	Control	37.39	(7.36)	37.29	(7.16)	-0.26	0.52	0.2
	Treatment	40.25	(8.54)	40.35	(8.65)	0.23	0.54	

BMD (bone mineral density), BMC (bone mineral content

Conclusions

The improvement of total body BMC, total body BMD, BMDs of spine, femoral neck and hip were statistically significant among vitamin D treated patients compared to patients in the control group. Six months after stopping treatment the improvement in the regional BMD remained unchanged while only a marginal loss was observed in total body BMD and BMC.

Discussion

Most striking outcome of this randomized double-blind placebo controlled clinical trial conducted among patients with diabetic nephropathy was the significant reduction of urine microalbumin after monthly injection of vitamin D for six months. In addition, there was a significant reduction of serum creatinine and improvement of GFR among patients who received vitamin D. These results are supportive of the reno-protective effects of high dose vitamin D in diabetic patients with nephropathy who are on optimum medical therapy.

Further, vitamin D increased BMD / BMC compared to placebo given to patients in the control group. This improvement was observed in the total body BMD/BMC and BMDs of total hip, total spine and femoral neck. The regional BMDs remained unchanged six months after withdrawing vitamin D treatment while only a marginal loss was observed in total BMD and BMC.

Vitamin D caused no significant effect on cardiovascular risk scores, blood pressure or major serum lipid components except HDL.

In our sample we recruited patients in the early stages of renal disease (eGFR > 30 mL/min) and majority of them were not vitamin D deficient. Therefore we were able to increase their vitamin D levels to above physiological limits in order to examine for the non-classic benefits of vitamin D. These benefits were independent of the conventional treatment offered for these patients according to current treatment guidelines. According to the studies discussed above there was a significant reduction of urine microalbumin, serum creatinine and improvement of GFR after monthly injection of vitamin D for six months. These results are supportive of the reno-protective effects of high dose vitamin D in diabetic patients with nephropathy who are on optimum medical therapy. Furthermore, we observed a significant reduction of renin levels in the treatment group compared to the control group.

The dose of vitamin D used in this study raised serum vitamin D level substantially. Although this was sufficient to demonstrate reno-protective effect and benefit on BMDs the period of trail was insufficient to demonstrate a positive effect on CV measurements except HDL.

Based on the results of these studies vitamin D can be considered as an add-on therapy to patients with increasing microalbuminuria despite optimum glycaemic and blood pressure control and receiving maximum tolerable doses of ACEI or ARB.

Due to the paucity of data, however, further clinical trials should be done to reproduce the results observed in this study. If the same benefits are proven, use of vitamin D for complete suppression of albuminuria can be recommended.

Conclusions

Monthly injections of high dose vitamin D_3 has improved the renal functions, BMD and BMC in patients with diabetic nephropathy.

This treatment did not have a significant effect on cardiovascular risk scores or blood pressure except HDL levels.

Further studies involving longer durations of treatment at different doses of vitamin D may be needed to reconfirm these findings.

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Socioeconomic Status and Stress: Neuroendocrine Pathways to Disease

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ABSTRACT

The biomedical model investigates diseases of the organism at the cellular level, and ignores the broader socioeconomic factors that affect health. This discursive analysis examines the physiological mechanism by which socioeconomic factors get *inside* the human body and make people susceptible to diseases. A survey of recent materials was carried out using PubMed database to identify peer-reviewed manuscripts that examine physiological reaction to stress and its connection to diseases. These studies reveal a complex relationship between socioeconomic status and chronic diseases.

Key words: *Stress, adaptation, allostasis, allostatic load, illness*

Introduction

During the last three decades, researchers have been able to establish the physiological connection between socioeconomic status and diseases such as hypertension, coronary heart diseases, stroke, type II diabetes, memory impairment, and malignancies, which seem to increase as we go down the socioeconomic ladder of a society. While the social gradient may explain the continuous distribution of risks and vulnerabilities, the fundamental question that these researchers have been trying to answer was how does socioeconomic status get *inside* our bodies and make us susceptible to diseases? This discursive analysis, based on the current literature, explains the physiological mechanism through which socioeconomic status manifests as a major cause of disease, and makes some policy recommendations to mitigate the socioeconomic challenges, and their health damaging consequences.

Understanding the Social Determinants of Health:

The social determinants of health include a wide range of interrelated socioeconomic factors that affect people's everyday living. For example, income and employment status, the level of education, early childhood experience, and access to healthcare are some of the important social and economic factors that affect health throughout life. Yet, almost universally, these factors are not adequately incorporated into health policies and practices. Health has long been regarded as an outcome of good medicine. Although modern medical breakthroughs have eradicated many infectious diseases, the level of *exposure* to disease risks is often dependent upon a wide range of socioeconomic factors. It is only recently that policy makers have begun to examine population health within the broader context of socioeconomic status, and to introduce population health promotion strategies as part of the overall socioeconomic policies.

The social and economic problems influence health in every stage of life. For example, recent studies have shown that low birth weight and emotional deprivation in childhood are major causes of learning disabilities and other behavioral problems, which could in turn be a precursor to long-term material disadvantages in adult life. Moreover, these

material disadvantages are both absolute and relative: for example, not having money to buy food, or a place to live is a measure of an absolute poverty, which is a common problem among the people of the lowest rung of the social hierarchy. The effect of absolute poverty during in-utero growth through early childhood is known to produce biological markers for many adulthood diseases, such as diabetes and coronary artery diseases (1).

Relative poverty, on the other hand, is the difference between the standard of living enjoyed by the people in upper social echelons and those in the lower social classes in the same society. Relative poverty is believed to affect health through psychological and other status symbols. Although absolute poverty has significantly declined in recent years, relative poverty is rising throughout the world due to widening income gap between the rich and the poor. Studies have shown that in societies where socioeconomic disparities are higher, the overall mortality rate, infant mortality, violent crimes, hostility, obesity, and interpersonal distrust are greater (2). Thus, health is powerfully affected by both absolute and relative socioeconomic status. As we go down the social ladder within a given society, morbidity and mortality rates increase because the same people suffer from both types of deprivation. How does socioeconomic status get "under the skin," and make people sick?

Adaptation to Socioeconomic Pressure:

The link between socioeconomic status and disease is "stress," the term that often used to express the physical and psychological "pressure" exerted by various external challenges. These external challenges, which include socioeconomic problems, are powerful stressors in life. However, the impact of these stressors varies depending on the length of exposure to such challenges, the availability of supportive resources, and the individual genetic factors. Bruce McEwen defines stress as "a threat, real or implied, to the psychological or physiological integrity of an individual" (3). He recognizes, however, the ambiguity of the term as it is used in everyday life, which makes it difficult for us to understand how the human body copes with stress. As researchers have pointed out in recent years, this coping mechanism, which is described as *allostasis,* enables the organism to adapt to the external challenges, so that it can maintain the internal functional balance (*homeostasis*) of organ systems, and sustain life. Any physical or psychological challenge disturbs the functional balance of organ systems, and the body reestablishes it by adjusting neuroendocrine adaptation. Humans have survived on the earth by adapting to their physical and social environments. Overtime, however, the coping mechanism increases the vulnerability to major diseases, which is described as the *allostatic load*, or the cumulative wear and tear on the organ systems and their tissues due to prolong, insufficient, or failed adaptation (4).

As the brain determines the nature of the challenge, it regulates neuroendocrine response. Coping with any acute challenge requires extra energy for physical and psychological endurance. Thus, the autonomic nervous system, via the hypothalamic-pituitary-adrenal (HPA) axis, triggers the *catabolic* function of the digestive system resulting in an increase release of energy by breaking down protein, carbohydrate, and fat storages in the muscles and the liver. The release of three major endocrines (hormones), such as *epinephrine*, *norepinephrine,* and *dopamine* (collectively known as *catecholamine*) from the adrenal medulla increases the supply of blood to the heart, brain, and skeletal muscles. This in turn triggers fundamental physiological reactions affecting blood pressure: 1) the release of *epinephrine* triggers the discharge of the *glucagon* hormone by the pancreas to breakdown *glycogen,* the energy storages in the liver, which increases the plasma levels of glucose, free fatty acids, low-density lipoprotein, and cholesterol to provide more energy; 2) the discharge of *norepinephrine* stimulates the heart muscles to increase cardiac output (the heartbeat), and vasoconstrictions (tightening of the arteries) to eject more blood to the brain and skeletal muscles, and less blood to the gut and kidney; 3) *norepinephrine* stimulates the release of *renin-aldosterone* hormone in the kidney, which increases the plasma sodium concentration and arterial blood pressure. The *renin-aldosterone* mechanism increases the retention of sodium fluids in the blood flow, and prevents releasing the liquid through urine; 4) *dopamine*, primarily a neurotransmitter from the brain and autonomic nervous system, regulates the blood flow through the arteries, and the secretion of both *epinephrine* and *norepinephrine.* With the rising cardiac output, fluid

volume, and vasoconstriction, the blood pressure rapidly elevates forcing more blood to the brain and skeletal muscles, while restricting the blood flow to elsewhere in the body (5).

As these neuroendocrine mechanisms intensify the functional output of several organ systems, the release of the *glucocorticoid* hormone from the adrenal cortex regulates the *catabolic* process that increases the blood level of glucose. Obviously, all these physiological and neuroendocrine reactions to an external challenge require a fundamental shift in the performance of a number of major organ systems from an *anabolic* state of repairing tissues, producing immune cells, stimulating reproductive hormones, and storing excess energy to a *catabolic* state in which more energy is provided to certain organ systems to intensify their activity, while suppressing the performance of others temporarily in order to sustain life (or to face the challenge).

Adaptation and Disease:

The stress induced disease, or *allostatic load,* is the chronic wear and tear on the body resulting from adaptation to stressor. As noted, a common outcome of the adaptation (*allostasis*) is cardiovascular reactivity resulting in high blood pressure. The elevated blood pressure accompanies several risk factors such as increased blood levels of glucose, low-density lipoprotein, and cholesterol, which are all known clinical risk factors for heart diseases, diabetes, obesity, and strokes. Studies have shown that once the neuroendocrine process raises the blood pressure, and maintains it at a higher level for an extended period, blood pressure tends to stay high even if the initial cause of the elevation no longer exists. The brain and arteries develop structural and functional remodelling as part of the adaptation. This remodelling, over time, particularly in connection with arterial constriction, causes damage in the inner lumen of the arterial walls, where atherosclerotic plaques begin to develop, obstructing the blood flow. At this stage, even if the fluid volume has become normal, the narrowed arteries with blockages are bound to cause heart attacks and strokes (6).

Also, the release of *glucocorticoid* hormone during stress undermines the production of white blood cells, which has several pathological consequences. During prolonged stress the suppressed immune system delays the healing of wounds and injuries, and most importantly exposes the organism to various pathogenic agents such as viruses, bacteria, and carcinogenic agents, which are normally removed from the body by white blood cells. The immune system, particularly the thymus gland and its ability to produce white blood cells, is mediated by *glucocorticoids* (7).

Likewise, under chronic stress, *glucocorticoid* impedes the insulin function that regulates the blood glucose level. The function of insulin is to remove the extra glucose from the blood and store them in the muscles, body fat, and the liver as *glycogen* to be utilized when the blood glucose level goes down. During stress, the increased demand for energy to meet an external challenge prevents this insulin activity resulting in abnormally high levels of blood glucose. While elevating blood levels of glucose and free fatty acids, and preventing insulin from storing them in the muscles and the liver, the *glucocorticoid* promotes the deposition of *glycogen* in the abdomen. It has been identified that abdominal obesity, as in Cushing's disease, as well as type I and II diabetes are triggered by chronic stress (8). A number of recent studies have substantiated these observations reporting that excessive metabolic disorders among low-income groups, such as African-Americans, are related to psychosocial stress of living in hierarchical societies (9). Kate Pickett and colleagues found that in the top 50 developed countries, adjusting for gross national per capita income, the distribution of income is *positively* correlated with a number of health indicators such as the percentage of obese people in the population, diabetes-related mortality rate, and the average daily per capita calories intake (10).

While the brain is the main organ that regulates the neuroendocrine response to stress, it is also subjected to major neurological remodeling and structural transformations due to prolonged stress. Both animal and human studies have shown that prolonged activation of *glucocorticoid* receptors of the brain increases the risk of protein plaques deposition on the neurons obstructing the chemical messengers (neurotransmitters). The increased blood flow to the brain also carries with them high levels of plasma protein that accumulates on the neurons over time, eventually destroying them altogether. The *amyloid hypothesis* suggests that the accumulation of *amyloid* plaques on neurons may be linked to Alzheimer's disease. Recent studies of type II

diabetes, which is connected to increased plasma level of *glucocorticoid* under chronic stress, found reduced hippocampal volume (shrinking of the hippocampus). They have also shown that the reduced hippocampal volume is associated with memory impairment and cognitive dysfunction, and a general relationship between the size of the hippocampus and spatial memory (11). *Studies based on* human neuroimaging have shown that prolonged stress and post-traumatic stress disorders produce drastic structural changes in the prefrontal cortex, hippocampus, and amygdala leading to chronic depressive symptoms and anxiety disorders (12).

The neuroendocrine reactions triggered by socioeconomic challenges inevitably impose undue pressure on the organ systems and their tissues in the long-term. Generally, it is constructive when the neuroendocrine reactivity is rapidly mobilized on the organ systems and terminated immediately. However, when the neuroendocrine reactivity is prolonged due to chronic stress, it undermines mental and physical health. How rapidly those neuroendocrine mechanisms are activated and are halted is dependent upon the availability of effective coping mechanisms and resources for individuals, families, and communities. Unhealthy coping mechanisms could further aggravate damaging health consequences.

Behavioural Adaptations as Coping Mechanisms:

The pathophysiological conditions resulting from the adaptation to stress are often exacerbated by a variety of harmful behavioural adaptations, such as smoking, drinking, overindulging, and the lack of physical activity, which are often perceived as coping strategies by some individuals. These behavioural adaptations have the same pathogenic outcomes for major organ systems, as do the physiological adaptions to stress.

By contrast, however, studies have shown that the availability of social support, trusting interpersonal relations, regular physical activity, and membership in community organizations, which have been collectively described as *social capital*, enhances the resilience of organ systems. Any supportive relationship that has an attenuating effect on psychophysiological stress may have a positive long-term impact on health (13). People with social networks receive both material and emotional support during stressful situations in their lives. Social connectivity and supportive environments are known to increase inhibitory signals to the HPA axis, which in turn raises the HPA activation threshold thereby minimizing the effect of potential socioeconomic challenges to physiological system. In other words, if the individual is confident that support is available at a time of "distress" such knowledge could prevent the full-blown stress response from the HPA axis. The individual differences in social integration and connectivity (social networks) could therefore modify the cognitive perceptions of external challenges, which may in turn moderate the neuroendocrine reactions to such challenges (14).

Conclusion: Policy Recommendations

In recent years, particularly in developed Western countries, public policies on population health promotion have increasingly been incorporated into major economic policies. There has been significant effort to reduce the social gradient in health through inclusive economic policies, which have the potential to increase prosperity, greater redistribution of resources, and the overall social cohesiveness. These policies are results of several well-recognized public inquiries into the impact of socioeconomic inequalities in some countries, and the global campaigns by various multilateral organizations, such as the World Health Organization (WHO) and the World Bank, to inform about the "health cost" of inequality and economic marginalization. For example, the report of the Donald Acheson Commission of the United Kingdom, and the WHO Commission on Social Determinants of Health advocate that population health strategies to be based on four major pillars to counter the potential impact of material disadvantages from early childhood through adulthood: 1) reducing poverty and income inequality through targeted redistributive welfare policies to give greater educational and employment opportunities for disadvantaged social groups; 2) preventing unhealthy life-styles and life course issues, such as smoking, drinking, and malnutrition to promote healthy development; 3) prohibiting discrimination based on age, gender and ethnicity in areas such as employment, education and housing to

ensure social justice and equality; 4) ensuring universal access to quality healthcare, particularly primary care (15).

The underlying philosophy that guides these broad policy recommendations is that good health for all can be achieved by providing a good "foundation" in life through guaranteed access to education, healthcare and employment. Life is a progression through stages, in that material disadvantages in early childhood that prevent getting a good education, undermines the labour market opportunities during adulthood. People who have experienced disadvantages in early childhood are at the greatest risk in the subsequent stages of life. Policies need to prevent people from experiencing disadvantages at the earliest possible stages of life, and they need to give priority to those who are already at a disadvantage in society. These are the people who suffer from both absolute and relative deprivations.

The social environments–at work, school, or neighbourhood–that provide a sense of belongingness, security and safety are known as "healthy environments" as they prevent marginalization and deprivation, and promote a sense of well-being. In such social environments, individuals are less likely to make unhealthy lifestyle choices. By contrast, environments, in which people feel excluded, abused and discriminated, are regarded as "toxic environments," where people feel less worthy of themselves, seek the comfort of health damaging behaviours, and engage in criminal activities. Therefore, national and local authorities, as well as public and private employers, and community leaders need to recognize the health implication of social integration in institutions, as well as, in communities and neighbourhoods. Policies that prevent discrimination, abuse and violence not only increase economic productivity, but also promote health and well-being. People who are more socially connected: 1) live longer; 2) are more likely to survive a myocardial infarction; 3) are less likely to experience a recurrence of cancer; 4) are less likely to suffer from infectious illness, than those who are less integrated to the community (16).

Likewise, access to healthcare is a basic human right that has been recognized by the World Health Organization. Specifically, the access to primary care is critical for preventing disease, immunizing children and providing prenatal care services that build the foundation for healthy living. Although health care services themselves focus on the clinical aspects of risks rather than the social determinants of exposure to risks, preventive health services protect people from diseases and premature death. Public policies must ensure that all citizens have guaranteed access to essential health services, so that an unforeseen illness would not prevent people from fully participating in education and employment resulting in life-long socioeconomic disadvantage. Public policies need to address the socioeconomic determinants of health before they manifest as major health problems. This is the challenge for both policy makers and political leaders.

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Tissue Micro Array; establishing a cost-effective tool for cancer biomarker research in Sri Lanka

Running title: TMA for cancer biomarker research in Sri Lanka

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ABSTRACT

Tissue micro array is a cost effective tool for cancer biomarker discovery and for the validation and external quality control in immunohistochemistry. It has not been utilized in Sri Lanka before, although widely used in cancer research centres world over. Scarcity of cancer biomarker research in Sri Lanka is partly due to the unaffordable cost of laboratory consumables including antibodies. TMA is produced using tissue cores from multiple tissue blocks. It reduces the cost and improves the consistency in immunostaining and adds validity to the assessment. In this brief report, we describe the technique of producing TMA and technical issues faced and how we could overcome them.

Key words: *TMA, cancer biomarkers*

Review

The concept of embedding tissue of different samples into one tissue block goes back to 1986 when the 'sausage' tissue blocks were developed for immunohistochemical assessment (1). The advantage of sausage block was that all of the tissue samples are treated equally during immunostaining and most sources of variation are eliminated which facilitates comparative studies. It was recommended for large scale inter-laboratory quality control processes. This concept was further developed and Tissue Micro Array (TMA) was designed to its current format by Kononen *et al* in 1998 (2). Now it is an invaluable research tool in cancer biomarker discovery.

TMAs are paraffin wax blocks (recipient blocks) constructed with tissue cores extracted from multiple tissue wax blocks (donor blocks). TMAs are sectioned and histology slides are prepared and can be stained with any routine histological stains and immunohistochemistry. It is a high-throughput technology useful in histology based laboratory tests and can be used in florescent in situ hybridization as well (3). TMA can also be used to assess molecular parameters (DNA, RNA) by molecular techniques. While TMA is made, a template per block is prepared indicating the reference number to map the clinical details of the patient with the biomarker score. Once made, TMAs can be used for subsequent assessment of multiple markers. Therefore, TMAs can be used as tissue libraries for future research. TMA cuts down on the cost for antibodies and reagents by many folds as a small core of representative tissue is carefully selected instead of a routine tissue sample. The selected size of the core can be 0.6 to 2 mm. Therefore, a TMA block can be built with hundreds of tissue cores minimizing the variation that can occur during staining procedures improving the validity and increasing the cost-effectiveness. In this brief report, we intend to describe our experience in how this technique can be established in a routine histopathology laboratory.

TMA blocks can be constructed manually or by using precision instruments. Automated forms of tissue micro arrayers are also available but less cost effective for a country like ours. In our histopathology laboratory we used a TMA Builder (Thermo Fisher™) and manually constructed TMAs for a research project on immunohistochemical biomarker assay for a cohort of breast cancer patients who's clinic-pathological and survival details were available for mapping and subsequent analysis.

Making a TMA block

The TMA Builder consists of a mould and a punch extractor. The base of the mould has 24 pins which makes 24 pits in the recipient block. The mould-top has an inset for C-ring and two lifting screws. (Figure 1)

Paraffin wax pellets were melted at 60 ^{0}C in an oven to bring to liquid state. The base of the TMA mould was placed on a flat surface and a plastic C-ring was fitted into the inset in the mould-top. Molten wax was poured slowly to fill the C-ring which was then left to solidify. Once wax was solid enough, the C-ring filled with wax was removed from the metal mould by screwing down the two lifting-screws. The prepared wax block now has 24 pits to receive 24 tissue cores.

The donor tissue blocks were first examined for its physical suitability. The haematoxylin and eosin (H&E) stained slides of each case were reviewed. The best representative tumour region with minimum fixation artifacts was selected and marked for tissue extraction. The slide was superimposed on the corresponding donor block to identify the area in the tissue to be punched.

From each of these donor blocks, a core of 2 mm diameter tissue was extracted using the punch extractor of the TMA Builder™ (Thermo Fisher). The cores were transposed/injected into the pits in the recipient TMA wax mould prepared previously.

A core of brain tissue from a wax block was transposed into the 24th pit in the mould as a guide to identify the rows and the columns of the TMA. A template for each TMA block was prepared to link the biomarker score to clinico-pathological data of each case. We made 53 such TMA blocks containing breast cancer tissue of 1200 patients. Since the diameter of each tissue core was 2 mm which covers a sufficient surface area, tissue cores were not taken in duplicate (4).

TMA block was labeled in accordance with the template and was kept in the oven with the wax surface with tissue cores facing down on a flat metal surface. Temperature was set to 58 ^{0}C and left for 15 minutes to anneal the block and to bring the tissue cores to the cutting surface. Sections were cut at 4µ on a traditional microtome. Slides were kept overnight in the incubator at 60 ^{0}C before staining was done. Sections were assessed by light microscopy. TMAs also can be digitally scanned and displayed on a high resolution monitor (4). Scoring of the biomarkers on TMA was done blinded to the clinico-pathological data reducing the potential for bias. We were able to link this data to survival outcome and were able to prove the prognostic significance of immunohistochemical assessment of biomarkers in breast cancer which included new biomarkers (5). There are no other published reports on TMA technique being used for cancer research in Sri Lanka.

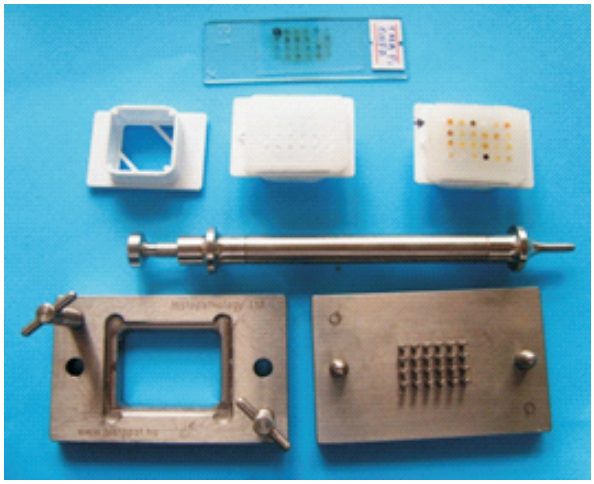

Figure1: This shows (items from top to bottom and from right to left) 1. A TMA slide stained for EGFR antigen; black circle indicating the guide core, 2.C-ring, 3. A recipient TMA paraffin block with 24 pits built on a C-ring, 4. A TMA block containing 24 tissue cores, 5. Punch extractor, 6. Mould-top of the TMA Builder with inset for C-ring and two lifting-screws in place, 7. Base of the TMA Builder with 24 pins.

Problems identified / troubleshooting

1. Locating the correct area to extract in a core biopsy donor block was difficult. The shape of the core of tissue which appeared on the block and matching it with the corresponding slide was used as a guide.
2. Since it is important to leave some diagnostic material in the block as archive, many core biopsies had to be excluded from our study. This was a limiting factor in preparing TMAs from core biopsies.
3. The depth to which the punch should cut into the donor block has to be first determined by trial as the extracted cores should be of the same length to fit into the pits in the recipient blocks.
4. Some tissue blocks were already sectioned extensively for the diagnostic process leaving only a thin piece of tissue and wax. The cores obtained from such blocks were very short compared to the depth of the TMA pit. The problem of shorter cores not reaching the cutting surface of the recipient block was resolved by keeping the TMA blocks in an oven as described in the annealing process. However, tissue loss was observed as such cores wore off after a few sections were obtained.
5. When the tissue in the recipient block contains fat around the tumour, correct superimposing of the slide to mark the correct site for core extraction was difficult. Inked resection margins, if available, were of help in such situations.
6. Breaking off of the outermost column of the tissue cores was frequently observed when the TMAs were sectioned. This occurred when the TMA blocks were fixed to the microtome through the plastic wings of the C-ring. The problem of breaking of blocks was overcome by fixing the TMA block through the frame of the C-ring.
7. Applying ice cubes on the surface of the TMA block just prior to sectioning, further reduced breakage of blocks.
8. Overnight incubation of tissue sections at 60 ^{0}C in the hot air oven, prior to immuno-histochemical staining, minimized the loss of tissue cores. This does not replace the necessity for a good section adhesive or a charged slide. Loss of tissue cores was minimal with H&E staining. When the guide core of the tissue section was lost during immunohistochemical staining, the H&E stained slide was very useful in identifying the location of the guide core. Therefore, it is advised to have the first section to be stained with H&E.
9. Folding of tissue sections was encountered at times. This occurred when the sections were too thick. Good microtomy skills were of utmost importance in obtaining sections of correct thickness without folds or cracks.

We believe that the information given in this brief report will be of value for Sri Lankan Histopathologists who wish to research into tumour biology and validate biomarkers using the plethora of cancer specimens they report and to find new knowledge on biomarkers at an affordable cost. It will be also useful in establishing external quality control system for immunohistochemistry laboratories in the country, which is a long-felt need.

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Compliance with Ethical Standards

This research project was granted approval from the Ethical Review Committee of the Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka.

Conflicts of interest

Author SNG received a monthly stipend as research assistant from the funding authority. The other authors declare that they have no conflicts of interest. The funding agency had no involvement in the study other than providing sufficient funds to conduct the research.

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An unusual cause of periodic limb paralysis "Gitelman Syndrome"

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Introduction

Gitelman syndrome (GS) is an autosomal recessively inherited salt loosing tubulopathy with a prevalence of 1-10 per 40,000 people (1, 2). The prevalence of GS is higher in Asia than other countries (2). GS is characterized by hypokalaemic metabolic alkalosis, hypomagnesaemia and hypocalciuria (3). It is typically seen in late childhood or adulthood. Symptoms are related to the degree of electrolyte disturbance. Cramps of the limbs are present among almost all and fatigability, polyuria, polydipsia, chondrocalcinosis are the other reported symptoms (1, 4). Clinical manifestations are less pronounced in heterozygotes (1).

We report a case of GS presented with a hypokalaemic periodic quadriparesis.

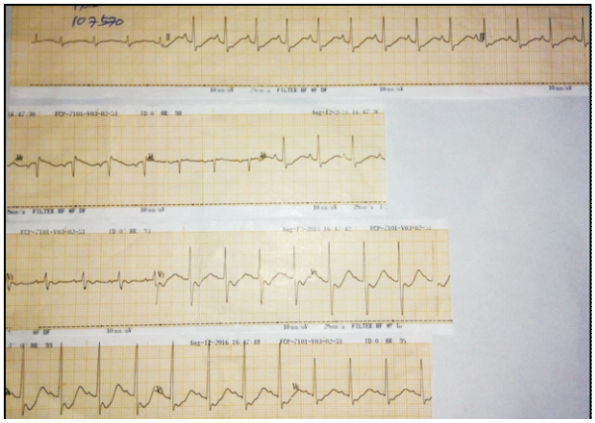
Case report

A 15-year-old previously healthy school boy noticed bilateral upper limb and lower limb weakness when he got up in the morning. Two days prior he experienced similar kind of weakness which resolved spontaneously. Weakness was not ascending and he denied dysphagia or breathing difficulty. His urinary and bowel habits were normal and there was no history of trauma.

Examination revealed flaccid quadriplegia with muscle power Grade 3/5 in upper limbs and Grade 2/5 in lower limbs. Power of neck muscles was normal and cranial nerve examination was unremarkable. All the reflexes were diminished and plantar response was normal. There was no sensory impairment. He was neither dyspnoea nor tachypnoea. Single breath count was more than 20. His pulse rate was 100/ minute and blood pressure was 135/65 mmHg. Respiratory system and abdominal examinations were unremarkable.

Evaluation revealed serum K^+ 2.1 mmol/L (3.5 - 5.3), serum corrected calcium 2.59 mmol/L (2.1 -2.57), serum magnesium 0.68 mmol/L (0.66 - 1.07), pH 7.42 with HCO_3 28 mmol/L, 24 hour urinary calcium excretion 0.2 mmol/day (2.0 - 7.5 mmol/day) urinary excretion of sodium 126 mmol/L, urinary potassium excretion 112 mmol/L and urinary chloride excretion 140 mmol/L, supine aldosterone 343.2 pg/mL (49.3 - 175) and supine renin 36.1 pg/mL (2.7 - 32.6). Parental screening revealed asymptomatic hypokalaemia. (Mother - 3.4 mmol/L, Father - 3.1 mmol/L)

Intravenous potassium chloride (KCl) was commenced to correct the serum potassium deficit followed by oral KCl one tablet twice daily and he showed a remarkable response. Later he was started on spironolactone and KCl was tailed off. He was asymptomatic on subsequent clinic visits and serum potassium remained normal.



ECG on admission showing U waves and prolonged QT interval suggestive of hypokalaemia

Discussion

GS was first described in 1966, and its genetic basis was elucidated 30 years later. GS is unarguably the most frequent inherited tubulopathy and autosomal recessive in inheritance (5, 6). In the great majority GS is caused by mutations in the *SLC12A3* gene, which encodes the renal thiazide-sensitive sodium-chloride co-transporter (TS-NCC) that is specifically expressed in the apical membrane of cells in the distal convoluted tubule (DCT) (7).

Reduced NCC activity mimics the effects of persistent thiazide diuretic action, which include volume contraction, reduced or normal blood pressure, increased renin activity and aldosterone levels, renal potassium wasting and hypokalemia, renal magnesium wasting and hypomagnesaemia, and reduced urinary calcium excretion (1).

Impaired sodium chloride reabsorption leads to mild volume depletion and activation of the renin-angiotensin-aldosterone system (1). The combination of secondary hyperaldosteronism and increased distal flow and sodium delivery enhances potassium and hydrogen secretion at the connecting and collecting tubules leading to hypokalaemia and metabolic alkalosis (1). Hypocalciuria occurs due to loss of activity of TS-NCC which increases tubular reabsorption (3).

The diagnosis of GS is based on the clinical symptoms, biochemical abnormalities and largely one of exclusion with combination of characteristic set of metabolic abnormalities which includes hypokalaemia, hypomagnesaemia, metabolic alkalosis, secondary hyperaldesteronism, reduced urinary calcium excretion and increased urinary excretion of sodium and chloride (7,1). Other tests, such as genetic testing and measurement of the change in fractional excretion of chloride in response to loop and thiazide diuretics, are not widely performed (1).

Our patient exhibited clinical and almost all the laboratory criteria with the asymptomatic hypokalemia in both parents supporting autosomal recessive inheritance. Tubular defect of the GS cannot be corrected. There for treatment is life-long and is aimed at minimizing the effects of the secondary increases in renin, and aldosterone production and at correcting the volume deficit and electrolyte abnormalities. Genetic counseling is utmost important as the recurrence risk for parents with an affected child is 25% (7).

In general, the long-term prognosis is excellent and progression to renal insufficiency is extremely rare in GS (7).

Conclusions

Gitelman syndrome is one of the rare causes for hypokaleamia which is treatable and has excellent long term outcome. We achieved a good clinical and biochemical response with oral KCl followed by spironolactone.

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Differentiating scrub typhus meningoencephalitis, from tuberculous meningitis: Two case reports and a review

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Introduction

Scrub typhus is a zoonotic disease where currently about 1 million new cases are identified annually and 1 billion people may be at risk of this disease (1-3). The infection is acquired through agricultural activities in the rice fields, oil palm, rubber plantation and during recreational activities in the woods or mountainous areas which are common in Southeast Asia (4). Scrub typhus is underdiagnosed due to its nonspecific clinical presentation and lack of diagnostic facilities. The clinical manifestations of the disease range from sub-clinical disease to fatal organ failure (5) and it is commonly observed in endemic areas as one of the causes of fever of unknown origin (6). The pathognomonic clinical sign of scrub typhus is the presence of an eschar (60%) (7-9), which may be hidden and painless as it is often present in areas like breast folds, groin, external genitalia and gluteal folds which may go unnoticed in dark-skinned individuals.

The complications of scrub typhus usually develop after the first week of illness where jaundice, renal failure, pneumonitis, ARDS, septic shock, myocarditis and meningoencephalitis are all described. Central nervous system (CNS) involvement is a well-known complication of scrub typhus ranging from aseptic meningitis to frank meningoencephalitis (10). Patients with scrub typhus with meningitis and/or encephalitis present as confusion, agitation or seizures. Focal neurological signs are rare but are known to occur (11). Cranial nerve deficits are seen in ~25% of patients with the sixth being the most commonly involved (12). Unilateral or bilateral abducent palsies occur with or without meningitis and facial palsies ensue singly or in association with Guillain Barre Syndrome (13). Although focal CNS damage is rare complications like cerebellitis (14), myelitis (12), and cerebral hemorrhage (15) are reported during the encephalitis stage. Generalized cerebral vasculitis caused by scrub typhus (*Orientia. tsutsugamushi*) can present as meningitis or meningoencephalitis in 5.7 - 13.5% of patients. Cerebrospinal fluid (CSF) profile may show changes similar to viral meningitis such as mild leukocytosis, slightly increased protein content, and normal glucose levels (6). It should be included in differential diagnosis of aseptic meningitis and encephalitis in patients in endemic areas especially when alternative diagnoses are uncertain (5,11,16). In some instances features similar to tuberculous meningoencephalitis (TBM) such as cranial nerve palsies with CSF showing lymphocytic pleocytosis, moderately elevated protein levels, low glucose and elevated CSF adenosine deaminase (ADA) levels have been described (10).

To confirm the diagnosis, Weil-Felix test can be used with the Proteus OXK strain with a minimum positive titer of 1 : 80 or a fourfold rise over previous levels. Several studies have shown that the Weil-Felix test has a high specificity. It was found that at a cut-off value of $\geq$ 1 : 320, OXK had a specificity of 97% but it was less sensitive. Other tests that can be used to diagnose scrub typhus with higher specificity include indirect immunofluorescence test (IFA), immunoperoxidase test (IPT) and

complement fixation test (CFT). Also detection of scrub typhus IgM by ELISA method also has a high specificity (~90%) and sensitivity (~90%) when compared with IFA and IPT (13).

This paper describes two cases of scrub typhus presented as isolated meningoencephalitis with significantly elevated CSF proteins and completely recovered with appropriate antibiotics.

Case report - 01

A 47-year-old previously healthy male presented with generalized severe headache associated with photophobia and vomiting for 2-weeks. Initially he had high grade fever lasting for 7 days with arthralgia and myalgia. He did not have a history of altered sensorium, seizures. On examination, there was conjunctival congestion and neck stiffness but there was no mucosal pallor, jaundice, lymphadenopathy, rashes or oedema. Vitals were stable with respiratory and cardiovascular system examination being normal and he didn't have hepatosplenomegaly. Optic fundi were normal. Investigations showed a total white cell count of 6700 cell/mm^3 with normal haemoglobin, and platelets. His ESR was 36 mm and CRP was 4 mg/L. His renal and liver profiles and chest radiograph were normal and the peripheral smear for the malarial parasite was negative.

A lumbar puncture revealed a significantly elevated protein level of 153 mg/dL with 5 polymorphs and 30 lymphocytes per mm^3 with glucose of 68 mg/dL (plasma glucose 110 mg/dL). His CSF culture and bacterial antigen tests for pneumococcal, meningococcal and haemophilus spp. were negative. A clinical diagnosis of partially treated bacterial meningitis was made and patient was started on intravenous ceftriaxone. Despite continuation of ceftriaxone for more than 48 hrs the patient had continuous headache and fever reappeared during hospital stay. MRI brain was normal and CSF PCR for mycobacterium tuberculosis was negative. Upon further questioning patient revealed that he had been working in his lands visiting wooded areas prior to the onset of current illness. A through physical examination revealed a healed eschar in his groin that was unnoticed even by the patient until then (Figure).

A clinical diagnosis of typhus meningitis was made and patient was promptly commenced on oral doxycycline which resulted in dramatic symptomatic improvement resulting patient being afebrile and headache free within 48 hrs of treatment. Weil-Felix agglutination test was positive (PROTEUS OX 19 - 1/160, PROTEUS OX 3 - 1/160, PROTEUS OX K - 1/640). Repeat lumbar puncture performed after one week showed dramatic reduction of protein to 58 mg/dL and clearance of cells with normal glucose levels. Patient recovered completely and was discharged after completion of ten days of antibiotics.

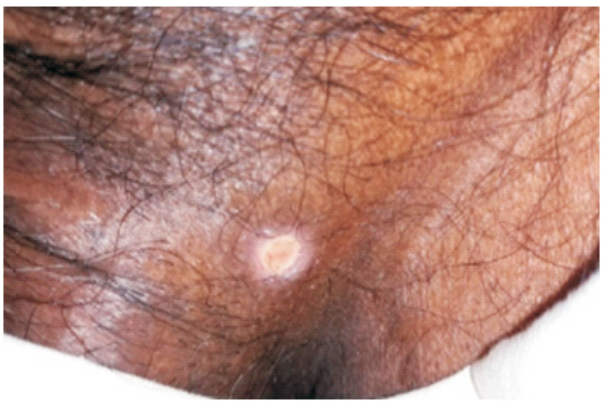

Figure: Eschar found in left groin (Case 1)

Case report - 02

Previously healthy, 23 year old male presented with history of fever for nine days and acute confusion with change in behavior. He was suffering from a dull aching type headache associated with vomiting without photophobia. One week prior to the admission he had participated in a mountain hike. On admission he was disoriented with a GCS of 14 (E - 4/4, M - 6/6, V 4/5). He was febrile (102 °F), tachycardic (pulse rate 108/min) with a blood pressure 110/70 mmHg. Neck was supple. On careful examination a maculopapular rash involving upper limbs, back and chest was noted. No eschar could be detected. On clinical suspicion of meningoencephalitis intravenous ceftriaxone was initiated. Full blood count showed elevated white cell count with neutrophil predominance. (WBC 11,300 /mm^3, Neutrophils 65%). CRP was 97.4 mg/L while ESR was 41mm. Blood cultures were negative.

Patient underwent lumbar puncture which showed CSF proteins of 157.5 mg/dL with a lymphocyte count of 2/mm^3. Polymorphs were not seen. CSF sugar was 60 mg/dL. (Random blood sugar 98 mg/dL). CSF culture, gram stain and antigen for common bacterial pathogens (pneumococcal, meningococcal and haemophilus spp.) were negative.

After 48 hours, patient continued to be symptomatic with high fever and the possibility of typhus meningoencephalitis was considered. Weil-Felix agglutination test showed positive results with PROTEUS OX 19 - 1/80, PROTEUS OX 3 - 1/160 and PROTEUS OX K - 1/320). Rapid test for scrub typhus antibody (IgM ELISA) also was positive. Oral doxycycline was initiated, with resultant dramatic clinical improvement within 24 hours and subsequently patient was discharged after 10 days of antibiotics.

Discussion

In the two cases reported we faced a diagnostic dilemma due to the elevated CSF proteins with lymphocytic pleocytosis and poor response to conventional antibiotics. Literature mentions that CSF in typhus meningoencephalitis can show a spectrum of changes. It may show leukocytosis, elevated protein, and slightly reduced glucose resembling viral meningoencephalitis, leptospirosis, and in certain instances tuberculous meningitis (6). Pai Het *et al*, reported a series of 25 patients with scrub typhus without signs of overt CNS involvement and showed that 48% had a reactive CSF showing mild mononuclear pleocytosis and PCR for *O. tsutsugamushi* was positive in 24% of cases. CSF white cell count ranged from 0 to 110, and the mean lymphocyte proportion was 51.9%. The CSF protein level was high (>50 mg/dL) in seven patients in this case series but only one patient had protein level more than 100 mg/dL (15). Another case series of 13 patients with suspected typhus meningitis / meningoencephalitis, mean CSF protein of 152 ± 66.88 mg/dL, glucose concentration of 55.23 ± 12.7 mg/dL and cell count of 46.07 ± 131 cells/mm^3 were seen. Most patients had lymphocytic pleocytosis and mean lymphocyte percentage was 98.66% ± 3.09% (17). Abhilash *et al,* reported a series of 189 patients with scrub typhus admitted with meningitis / meningoencephalitis (with IgM-ELISA positivity and/ or the presence of a pathognomonic eschar with PCR confirmation) (17), where mean CSF white cell count was 80 ± 120/mm^3 (range 5 - 900 cells) and > 60% patients showing counts up to 100 cells/mm^3. Only 10.5% (20/189) patients had counts exceeding 200 cells/mm^3 with lymphocyte predominance in 87.6%. The mean CSF protein level in this study group was 105.9 ± 80.9 (range 13 - 640 mg/dL) and was more than 100 mg/dL in 38% (72/189) of patients. This makes differentiating tuberculous meningitis (TBM) from typhus meningoencephalitis on CSF findings difficult. TBM is known to have high CSF proteins (>100 mg/dL) with lymphocytic predominance.

Although we have not performed, CSF Adenosine Deaminase (ADA) is another investigation that can be used in this setting. Some studies looked at ADA to differentiate TBM from typhus meningoencephalitis. CSF ADA >10 U/L increases the post-test probability of TBM, especially in a setting where TB prevalence is low. CSF-ADA sensitivity as high as 92.5% and specificity of 97% for the diagnosis of TBM at the cut-off at > 10 U/L has been observed (18). In a retrospective study in India (12) involving 65 cases of scrub typhus of which 17 had meningitis, CSF ADA levels were >10 U/L. In another study by Jamil *et al* (16), where CSF analysis was done in 13 patients of scrub typhus with clinical suspicion of meningitis / meningoencephalitis, 9 had ADA levels > 10 U/L. Mean CSF ADA was 16.98 ± 7.37 U/L with the highest ADA level of 33.25 U/L.

Other investigations which may be beneficial in these patients would be the detection of acid-fast bacilli (AFB) in CSF and Xpert-TB-PCR. Staining for AFB in CSF has low sensitivity and CSF culture for AFB take up to 8 weeks and is positive only in 50 - 75% of cases (19). Most studies on TB PCR (Xpert) found low sensitivity in detecting TBM (about 50%) and specificity was as high as 98% (20-23).

Scrub typhus meningitis can also be differentiated from TBM by the shorter period taken for the normalization (24) of CSF and the dramatic response to doxycycline. Rifampicin is also used to treat severe scrub typhus and in fact gives rise to better inhibitory concentrations in CSF (24) than doxycycline so that improvement following anti-

tuberculous therapy (ATT) may mask the diagnosis of scrub typhus.

We used doxycycline in both our patients and observed a marked improvement of symptoms within 24 - 48 hours. Rapid defervescence after antibiotics is so characteristic that it is used as a diagnostic clue for typhus as seen in our patient. Usually, the neurological abnormalities recover within 2 - 5 days of doxycycline therapy (13). In instances where doxycycline cannot be used other antibiotics such as tetracycline, azithromycin and rifampicin can be considered. Tetracycline 500 mg qid or doxycycline 200 mg once daily for 7 days is the treatment of choice. Azithromycin has been proven to be more effective than doxycycline in doxycycline-susceptible and doxycycline-resistant strains causing scrub typhus and can be used in pregnancy and in renal failure (11). Suggested mechanism for doxycycline resistance include poor CNS penetration with 15 - 30% of drug reaching the CNS, resistance, bacteriostatic nature, immune mediated injury etc. (16,25,26). Chloramphenicol 500 mg qid, is an alternative and as mentioned above rifampicin, 900 mg per day for a week has been found effective in patients who respond poorly to conventional therapy (27). Patients can be additionally given dexamethasone and mannitol if they have altered sensorium or cranial nerve deficits. In some instances, progressive neurological damage has been observed despite treatment calling for alternative medications (13).

Conclusion

Significantly elevated CSF protein levels with lymphocytosis can be observed in Scrub Typhus meningoencephalitis which can be confused with tuberculous meningitis. Recognition of an eschar or appropriate serological investigations will guide treatment specific for typhus. Rapid defervescence and faster normalization of CSF after doxycycline may be a clue to diagnosis.

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Critical limb ischaemia due to thrombosis of the right subclavian artery; a rare presentation of nephrotic syndrome

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Introduction

Thromboembolism is a well-known complication of nephrotic syndrome (NS) (1). The incidences of both venous and arterial thrombosis are higher in patients with NS compared to general population (1). Venous thrombotic complications have frequently been observed (2). Arterial complications are rare and may lead to serious and life-threatening complications depending on the vessels involved as well as associated with poor prognosis (2-5).

Hyperoaguability in NS is multifactorial, has been attributed to alterations in blood levels of factors involved in the coagulation and fibrinolytic systems, alterations in platelet function, venous stasis, haemoconcentration, increased blood viscosity and possibly the administration of steroids (6).

The deep veins in the legs, the inferior vena cava, the superior vena cava, and the renal veins are particularly involved among veins and there are case reports of cerebral venous sinus thrombosis (1,2). Though rare, involved arterial sites include aortic, renal, femoral, mesenteric, coronary, and cerebral arteries. The commonest site of arterial thrombosis is the femoral artery, occurring mainly in children with NS (5).

Here we report a case of critical limb ischaemia due to thrombosis of the right subclavian artery in an adult as a rare presentation of nephrotic syndrome.

Case Report

A 29 year-old patient with bronchial asthma and primary hypothyroidism presented with generalized oedema for one month, right upper limb intermittent claudication for three weeks followed by rest pain for two days. She was treated with diuretics for one month. There was reduction in urine output one day prior to admission. She was on oral contraceptive pills (OCP) for two years.

On examination there was generalized oedema with bilateral moderate pleural effusions and ascites. Right upper limb was cold with no radial, ulnar and brachial pulses with saturation of 68% on air (saturation of the left upper limb was 96% on air). Her pulse rate was 104 bpm and blood pressure was 120/70 mmHg. Rest of the examination was unremarkable.

Evaluation revealed nephrotic range proteinuria (6g/day) with bland urinary sediments, hypoalbuminemia (15g/L), hypercholestero- lemia (398 mg/dl) with evidence of acute kidney injury (Blood urea (BU) - 171 mg/dL, Serum creatinine (S, Cr.) - 206 μmol/L) and dehydration (Hb 17.1 g/dL, Haematocrit 57% with normal other cell lines) which improved with hydration (BU 92 mg/dl, S.Cr - 116 μmol/L, Hb 11.3 g/dL, Haematocrit 44%). Upper limb venous doppler revealed thrombosis of bilateral axillary veins with extension into brachial vein on right side. CT angiogram showed aberrant right subclavian artery with thrombosis of right subclavian, axillary and brachial arteries. Her autoimmune and thrombophilia screenings were negative.

She responded well to conventional heparin followed by subcutaneous enoxaparin and warfarin, high dose steroids, intravenous frusemide and intravenous albumin.

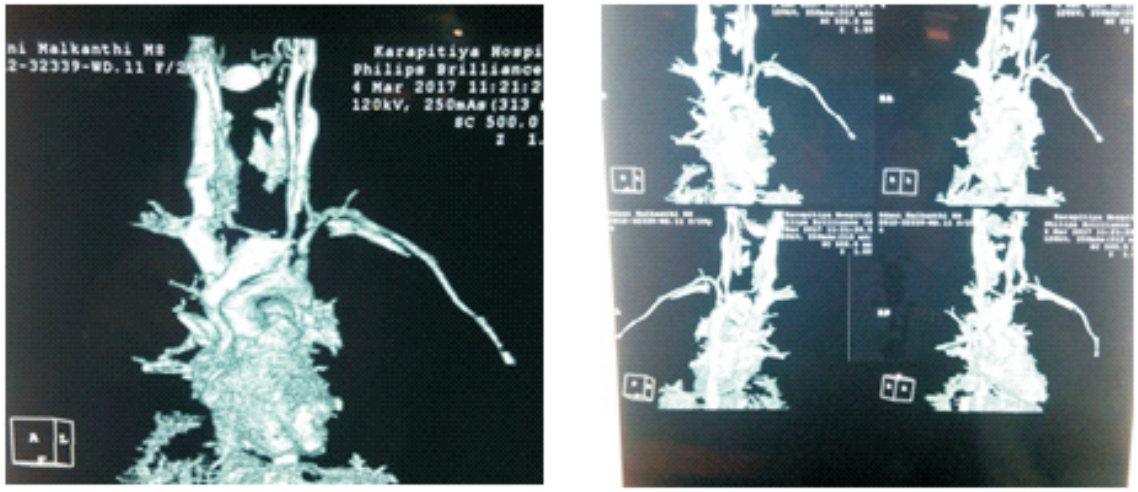

Figure: CT angiogram showing abrupt cut off right subclavian artery with thrombosis

Discussion

This 29 year old female presented with intermittent claudication of the right upper limb due to thrombosis of the aberrant right sided subclavian artery. At the same time she was diagnosed to have NS. In addition she was on OCP for 2 years and was dehydrated on admission. Thrombosis in general and arterial thrombosis in particular is a significant and potentially serious problem in patients with NS (5,8). Most cases of venous thromboembolism associated with NS reported in the literature have a preceding diagnosis of NS (5).

The increased propensity of thromboembolism in nephrotic patients is postulated to be a result of increased excretion of antithrombotic factors (antithrombin III, plasminogen, protein C, and protein S) by the affected kidneys and increased production of pro-thrombotic factors like fibrinogen by the liver (5).

Interestingly our patient had aberrant right side subclavian artery on which the origin is narrower than the usual one. She was on OCP and it is a well-known predisposing factor for thrombosis. Also she was dehydrated on admission without marked peripheral oedema with haemoconcentration probably due to pretreatment with diuretics. Hypercoaguable state of NS together with the effect of OCP, aberrancy of right subclavian artery and dehydration altogether contributed to thrombosis in our patient.

Arterial thrombosis can be diagnosed with the use of duplex scanning, CT angiography, or magnetic resonance angiography (6).

Once the diagnosis of is established, anticoagulation therapy should be started. Patient can be treated with conventional or low molecular weight heparin, followed by oral warfarin and antiplatelet agents. Thrombo-embolectomy or thrombolytic therapy is indicated in patients with ischemic limbs due to arterial thromboembolism. Attempts to reduce the degree of proteinuria, hyperlipidaemia, and hypertension should be made in all patients with NS (6).

The patient was treated with conventional heparin followed by subcutaneous enoxaparin and warfarin and remarkable improvement was noted.

To conclude, arterial thrombosis is a rare but a serious complication of NS at first presentation which needs high index of clinical suspicion for prompt diagnosis and immediate treatment is needed to prevent further complications.

Conclusion

Although rare both arterial and venous thrombosis can occur with NS. Hypercoaguable state of NS together with the effect of OCP, right subclavian artery aberrancy and dehydration have all contributed to thrombosis in this patient. We saved the limb due to prompt diagnosis and early treatment.

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Knowledge, attitudes and practices regarding modern methods of postpartum contraception among postnatal mothers

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ABSTRACT

Introduction: Improving postpartum contraceptive use is an important programmatic strategy to improve the health and well-being of women, newborns and children in a country.

The aim of this study was to assess the knowledge, attitudes and practices regarding postpartum contraception among postnatal mothers.

Method: A cross sectional study on 300 postnatal mothers was carried at Teaching Hospital Mahamodara (THM) Galle. Multiparous mothers were recruited using convenient sampling method. Data collection was done using a validated, self-administered structured questionnaire after obtaining informed consent. Questionnaire was designed to assess on knowledge, attitudes and practices of postpartum contraception.

Result: Level of knowledge regarding postpartum contraception was above average in 15.8% of mothers and below average in 46.6% of them. The majority of them were aware regarding Combined Oral Contraceptives Pills (COCP). Almost 60% of women of Islamic and Hindu religions believed that contraception is not accepted by their religion. Among postnatal mothers, 70.2% had previous practices of postpartum contraception and the commonest method used was COCP. Approximately 76% of mothers gained information regarding postpartum family planning from Public Health Midwives (PHM).

Conclusions: Knowledge on post-partum contraception in general was inadequate. The attitudes and practices on postpartum contraception were influenced by their religion. Short acting contraceptives were popular method of contraception among postnatal mothers. The major information provider was PHM.

Keywords: *Postpartum contraception, Knowledge, Attitudes, Practices*

Introduction

Fertility control with the use of contraceptives is essential to the health and well-being of individuals, family and community. It has been noted that demand fluctuates over the course of reproductive life. Childbirth changes priorities, attitudes and lifestyles of women. Postpartum period is particularly important because adequate birth spacing can improve maternal and infant health (1).

The majority of women resume sexual activity within several weeks of the delivery. The amount of time following delivery that a woman is infertile is highly variable and dependent on multiple factors, including breastfeeding status. Ovulation can occur even if the mother has not resumed menstruation and could happen as early as 25 days postpartum. The probability of ovulation occurring before resumption of menstruation increases over time (2).

Postpartum family planning (PPFP) focuses on the prevention of unintended and closely spaced pregnancies through the first 12 months following childbirth (3).

Globally unmet needs for contraception of women remain high despite of availability of modern safe and effective contraceptive methods. Yet unmet need is constituted a large percentage of total demand in many countries. Unmet need for family planning in Sri Lanka is 15% (4).

Unmet needs could lead to unplanned and unintentional pregnancies which will increase the risk of adverse maternal and neonatal health outcomes. Improving postpartum contraception is an important programmatic strategy to improve the health and well-being of women, newborns, and children (1).

Postpartum contraceptive advice allows women to plan the spacing and number of future children. Short inter-pregnancy intervals have been associated with an increased risk of adverse perinatal outcome; therefore aside from the socioeconomic benefits, delaying future pregnancies may be beneficial in terms of health (5). Postpartum women face uncertainty about timing of return to fecundity. Many women wait to use contraception until menses return, resulting in unintended pregnancies (6).

The choice of a postpartum contraceptive method depends on many factors, including the need for a temporary versus a permanent method, the infant feeding choice and the extent to which informed consent is made prior to delivery. For maximum protection, the non-breastfeeding woman should be protected from the fourth week postpartum, even if that means using a temporary method, such as condoms, until her method of choice is certain (7).

The postnatal period is associated with physio--logical, psychological and social changes, which can influence sexual and reproductive health. Although women may wish to delay or avoid further pregnancy, they may not know how to access contraception or which methods are safe to use, particularly if they are breastfeeding. There may also be difficulties with sexual function and relationships during this time, for which individuals may require information and support (5).

Postpartum contraception is a subject which has been discussed worldwide and it is very important for improvement of their economy. Nearly two-thirds of women in first postpartum year had inadequate contraception in United States of America, for example, nearly 60% of pregnancies conceived at any time are unplanned (23% unintended and 37% mistimed) (8). Surveys from 27 countries indicate that two-thirds of postpartum women had an unmet need for contraception (9).

Ninety five percent of women in low and middle-income countries wish to avoid a pregnancy within next two years, but 70% are not using any method of contraception (10). Common reasons for unmet need for family planning were inconvenience, unsatisfactory services, lack of information, fears about contraceptive side effects and opposition from husbands, relatives or others (11).

In Sri Lanka, the most common reason for illegal termination of pregnancy is youngest child being too young (12). This largely reflects the unmet need among postnatal mothers.

Unmet needs among Sri Lankan women remains approximately 7% among postnatal mothers (13). One of the most common reasons is lack of knowledge regarding different type of contraception methods. The aim of this study was to assess the knowledge, attitudes and practices regarding postpartum contraception among postnatal mothers.

Methods

A descriptive cross sectional study was carried out at Teaching Hospital Mahamodara. Mothers who were in immediate postpartum period were recruited for the study. Postnatal mothers who were not able to read, acutely unwell, suffering from psychiatric illnesses and in intensive care unit were excluded from the study. Three hundred consecutive postnatal mothers were recruited from four postnatal wards during the periods of three months from March to May 2015. Eight postnatal mothers were excluded from the study. Data were collected using a pre-tested self-administered questionnaire that included four sections to evaluate the knowledge, attitudes and practices regarding postpartum contraception among postnatal mothers. It consisted of both open and close ended questions. Section one was designed to obtain basic socio-demographic data. Section two consisted of 33 facts to assess their knowledge about postpartum family planning. Section three was designed with 20 facts to evaluate their attitudes regarding PPFP practices. Section four consisted of four subsections to assess previous practices regarding PPFP. Questions assessing knowledge, attitudes and practices were assigned scores using

predetermined cut-off values. One hundred points were allocated to 33 facts to assess their knowledge about postpartum family planning Postnatal mothers were categorized in to above average (score > 60), average (score 41 to 60) and below average (score < 40) on levels of overall knowledge. Ethical approval was granted by ethical committee of the Faculty of Medicine, University of Ruhuna. Permission for collecting data in hospital was obtained from the Director of the THM.

Participants were fully informed about the purpose of the study and obtained the written informed consent. The privacy and confidentiality of each participant taking part in study was ensured.

Data was entered in to a data base created using Statistical Package for Social Sciences (SPSS) version 20. Data analysis and result presentation were done using mean, p value, standard deviation (SD) and percentages.

Results

Table 1: Basic characteristics of the sample

Socio demographic characteristics		Percentage (n = 292)	
Age	20 -29	32.9%	(96)
	30 -39	61%	(178)
	40 -49	5.8%	(18)
Religion	Buddhist	89%	(260)
	Islam	9.6%	(28)
	Christian	1.4%	(4)
Ethnicity	Sinhala	88.4%	(258)
	Muslim	10.3%	(30)
	Tamil	1.4%	(4)
Level of education	Up to O/L (secondary)	43.8%	(128)
	Up to A/L (secondary)	38%	(111)
	Diploma (high)	4.1%	(12)
	Degree (high)	5.5%	(16)
	Under grade 10 (primary)	8.6%	(25)
Marital status	Married	97.9%	(286)
	unmarried	1.7%	(5)
	Divorced	0.3%	(1)
Income	Not mentioned	10.6%	(31)
	1 - 10,000	8.2%	(24)
	10,001 - 50,000	74%	(216)
	50,001 - 100,000	6.8%	(20)
	>100,000	0.3%	(1)
No of children	2	63.7%	(168)
	3	27.7%	(81)
	4	6.8%	(20)
	≤ 5	1.7%	(5)

According to the age distribution of the sample, majority (61%) was in 30-39 years age group. Almost 90% of mothers were Sinhala Buddhist. Approximately 80% of mothers have had educational level up to ordinary level or above.

Table 2: Overall level of knowledge on postpartum contraception

Level of knowledge	Percentage % (n = 292)
0 - 40 (Below average)	46.6% (136)
41 - 60 (Average)	37.7% (110)
> 60 (Above Average)	15.8% (46)

Approximately half of mothers had poor knowledge regarding postpartum contraception.

Table 3: Willingness of participants to use a contraceptive method after a child birth

Attitudes to wish to use contraceptives	Percentage (n = 292)
Yes	83.6% (244)
No	16.4% (48)

Out of 292 postnatal women more than 80% women wished to use some type of contraceptive after the child birth.

Table 4: Beliefs regarding post-partum contraception

	Yes (%)	No (%)
Delayed till next pregnancy	88.7 % (259)	11.3 % (33)
Not accepted by the religion	8.9 % (26)	91.1 % (266)
Sexual satisfaction is minimal	7.5 % (22)	92.5 % (270)
Fear of side effects	44.2 % (129)	55.8 % (163)
Doubts regarding instructions	5.8 % (17)	94.2 % (257)

Approximately 10% of mothers believed religion is a deciding factor for contraception and 45% mothers has had fear of using a contraceptive method due to its side effects.

A vast majority (70%) used some type of contraceptive method following their previous childbirth (within first six months).

Table 5: Percentages usage of different types of contraceptive methods after previous child birth

	Percentage (%)
Combined Oral contraceptive Pills	40.4% (83)
Injectables (DMPA)	20% (41)
Progesterone containing implant (Judella)	1.46% (3)
IUD (Copper T)	20% (41)
Male condoms	17% (35)
Female condoms	0% (0)
Emergency pills (Levonorgestrel)	0.4% (1)
Emergency IUD	0.4% (1)
Others	0.4% (1)

Almost 40% of mothers had used COCP as a postpartum contraceptive method following previous child birth.

Majority of women (76%) had gained their knowledge regarding PPFP from Family Health Workers. Approximately 10% of them acquired information from both doctors and nurses. Media helped to gather knowledge only in 2.7% of women.

Discussion

The study found that overall knowledge about PPC is generally inadequate among postnatal mothers. Vast majority of postpartum mothers wished to use some kind of contraceptive method to space-out next pregnancy. Two third of mothers have practiced PPFP following last child birth and among them most popular method had been COCP. Family Health Worker had been the major source of their awareness.

Even though we expected high level of knowledge among postnatal mothers, this study revealed inadequate knowledge regarding PPC.

When considering attitudes, vast majority of women (83%) wanted to have some type of contraceptive after child birth. But half of them afraid due to side effects of the contraceptive methods. Significant number of non-Buddhist women believes that contraceptive usage is not accepted by their religion. A 2010 analysis of Demographic and Health Survey data from 17 countries demonstrated that 50 - 88% of women in the first year of postpartum period would like to avoid pregnancy but are not using contraception. Policy efforts for providing family-planning services to postpartum women have primarily focused on the first six weeks after delivery, but the extension of services through the first year postpartum is likely to further improve birth spacing. WHO recommends an interval of 24 months or more before attempting a next pregnancy after a live birth, to reduce the risks of adverse outcomes for mother and child (14). This study shows that the majority of mothers did not know exact timing of using PPC although they were aware regarding contraceptive methods.

In our study 70.2% mothers had previous practice of postpartum contraception and the most popular method had been the COCP. According to Cooper *et al*, approximately one third of women were using a modern contraceptive method in his study (6). In the second decade of the 21st century, the best available figures suggest that the contraceptive pills and the condom remain the most widely used methods in Britain (9). LARC (long acting reversible contraceptives) has high acceptance rate as postpartum contraception in our study population following previous child birth. This is a healthy trend. Kari et al identified across states, that there was a wide range of use of female sterilization and long-acting reversible contraception. Practice of LARC increased $\geq$ 18% per year, while use of injectables and oral contraceptives declined by 2.5-10.6% annually (15). LARC should be encouraged by health care profession as most effective contraceptive method.

Of the mothers those who have had previous practice of postpartum contraception (n = 205), majority (93%) mentioned that the commonest sources information gained was from family health workers. This is probably due to family health worker being their first contact care giver in the community.

However we must encourage other health professionals to counsel mothers at different levels of contact to reduce unmet need of contraception.

Conclusions and recommendations

Knowledge on post-partum contraception in general was inadequate among postnatal mothers. Vast majority of postpartum mothers wished to use some kind of contraceptive method to space-out next pregnancy. Two thirds of mothers have practiced PPFP following last child birth and among them most popular method had been COCP. Family Health Worker had been the major source for their awareness.

We highly encourage health care professionals to counsel eligible couples and postnatal mothers regarding PPFP. This can be facilitated through setting guidelines by policy makers. Modern methods of contraception, specially LARC should be freely available in the family planning clinics for informed choice.

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Epidemiology of attempted suicides in Southern Sri Lanka

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ABSTRACT

Suicides are emerging as an important public health issue globally. Suicide rate in Sri Lanka is higher than the global figure and is around 20 per 100,000 population. Knowledge on characteristics of subjects who attempt suicide will help to strengthen prevention programs.

Therefore the aim of this study was to describe characteristics of patients who admitted following attempted suicides. A descriptive cross sectional study was performed using an interviewer administered questionnaire in General hospital Hambantota, which is a tertiary care institute in southern Sri Lanka.

Total sample was consisted of 105 subjects. Mean age was 25.5 (SD 11.93) and 56.2% were females. Except 4 subjects the rest were Sinhala Buddhists. Majority had only secondary education (71.4%), were not employed (55.2%) and belong to nuclear families (79.0%).

Though majority admitted an access to help (65.7%), had good relationship with parents (73.3%) and socially connected, there were 15.3 % with past history of suicidal attempts and 64.7% had exposed to suicidal acts in media. There were 6.7% with depression who were all females and 5.7% with alcohol dependence who were all males. Though 56.1% reported recent stressful life events, only 27.6 % had mentioned about recent disputes in a relationship.

Important outcomes

- Majority of the sample were females, from 16-25 age group, had only secondary education, not employed and belong to nuclear families.
- Larger proportion admitted access to help and socially connected, but majority had reported recent stressful life events.
- There were 6.7% with depression who were all females and 5.7% with alcohol dependence who were all males.

Important limitations

- A hospital based sample was taken for this study and hence led to selection bias and may not give the true picture in community.
- The ascertainment of outcome was totally depended on patient's declaration of the intent. Those who deliberately harmed themselves without a true intention of dying may give a different reasoning later.
- Association with assessed factors could be elaborated better if an analytical approach was taken.

Introduction

Suicides are emerging as an important public health issue worldwide. Every year an estimated number of 900 000 people die by committing suicide. This represents one death every 40 seconds (1). Worldwide incidence of completed suicides is 16 per 100,000 population and it ranks as the 13th leading cause of deaths, globally, according to current World Health Organization (WHO) statistics (2). Ten to twenty times more people attempt suicides than above incidence (3). This adds an immense burden to health care systems worldwide.

Sri Lanka became top ranked in the world by suicide rates in 1995 (47 per 100,000). Although there is a steady and a gradual declining of suicide rates since then, the rate recorded in 2008 was 20.4 per 100,000 of population (4). In 2008, 4120 people succumbed to suicides (3260 males and 860 females) according to the Department of Police data. Though we do not have accurate figures, it is assumed that nearly 40000 people attempt suicides each year. Therefore it is a major socio-economic problem (5) and is high enough to be considered as a top public health issue in the country.

Taking immediate actions to reduce the morbidity and mortality due to suicidal behaviour has become a top priority in WHO agenda. WHO has made a call for a co-ordinated and intensified global action to address this problem. Therefore area specific data is of utmost importance in planning and implementing prevention programs. Evidence based guidance for public health policy making is the key to reduce the burden of suicides.

Attempts to understand and prevent the occurrence of suicidal acts require the assessment of epidemiology of the problem in a given setting (6). Knowledge on characteristics of subjects who attempt suicide in a particular area will help to strengthen prevention programs and yield a better outcome.

Therefore the aim of this study was to describe characteristics of patients who were admitted following deliberate self-harm to a tertiary care institute in southern Sri Lanka.

Methods

Study was conducted in General Hospital Hambantota, a tertiary care institute in the Southern part of the country which caters for a population of nearly one million. A descriptive cross sectional study was performed using an interviewer administered questionnaire as the data collection tool. The data was collected from May to September in the year of 2011.

A person presented with a nonfatal act of self-harm undertaken with conscious self-destructive intent was taken as a case of suicide attempt (7). Ascertainment of attempted suicide was done according to the diagnosis made by the Medical Officer-Mental Health (MO-MH). In routine practice, every deliberate self-harm case is referred to MO - MH who is a postgraduate qualified medical officer in psychiatry who serves under the supervision of a consultant psychiatrist. The interview was performed when the patient was referred for psychiatric assessment by the MO-MH, therefore it was assumed that all the patients selected were physically and mentally capable of providing information. Those who were psychologically or emotionally disturbed or not in a state to undergo the interview were excluded. Eligible subjects were informed about the study and the interview was performed after obtaining written informed consent from the patients. Special emphasis was given to privacy, autonomy, non-maleficence and expected benefits.

Permission from hospital authority was obtained and minimal disruption to the routine patient care was assured. Ethical clearance for the study was obtained from ethics review committee of the Faculty of Medicine, Colombo.

Questionnaire used in the study was a self-constructed one. Selection of variables was done by triangulation of methods. Several text books in psychiatry were scrutinized to identify main aspects of epidemiology of attempted suicides (3,8). Thorough literature survey was done by referring relevant research publications based on the topic. Google scholar and PubMed were explored in finding relevant publications. Finally expert opinion was obtained with the objective of adjusting these variables to local setting. It was pretested with 10 cases in a General Hospital of an adjacent district and further modifications were made.

The questionnaire included background information and items to asses each variable. They were arranged

into a logical order to make the interview more comfortable and facilitate recalling events. Simple language was used. Vague and emotional words were avoided.

The questionnaire was originally developed in English and then translated into Sinhala and Tamil languages. Back translations were done to match with the original English version. Standard diagnostic criteria for research given in ICD 10 classification of mental and behavioural disorders by WHO were used for the assessment of depression and alcohol dependence.

Data were collected by the principal investigator and four research assistants who were former psycho-social workers served under a WHO guided program. To reduce observation bias the research assistants were trained by the principal investigator with special attention on maintaining uniformity. The principal investigator and research assistants were trained by a Consultant Psychiatrist on detecting the two psychiatric conditions assessed in the study using ICD 10 classification of mental and behavioural disorders by WHO.

Patients identified with any of the two psychiatric illnesses assessed were referred to psychiatric unit for appropriate management. Those who needed socio-economic support were referred for pre identified government officers, non-governmental organizations or community based rehabilitation program conducted by the government. Subjects who needed counseling were referred to counseling officers attached to Divisional Secretariat offices in respective areas.

Data analysis was done by SPSS software. Relevant descriptive statistics were calculated and comparisons were done to elicit statistically significant characteristics.

Results

The sample included 105 eligible subjects who agreed to participate.

Basic demographic characteristics of the study sample

Distribution of basic demographic characteristics of the study sample is shown in table 1.

The mean age of study subjects was 25.5 (SD11.9) years and the age range was from 12 to 70. Majority of them (57.2%) belonged to 16 - 25 age group. Females (56.2%) exceeded males in number. Except four subjects the rest of the study population were Sinhala Buddhists. Fifty eight subjects (55.3%) reported a monthly family income of Rs. 10,000/- to 30,000/- while 46 subjects (43.8%) admitted that they had below Rs. 10,000/- monthly family income. Majority of the sample had educated from grade 6 to 11 (71.4%), not employed (55.2%) and belonged to nuclear families (79.0%). Study population consisted of nearly equal portions of married and unmarried subjects (50.5% and 49.5%).

Surprisingly 65.7% of patients admitted that they have an access to help, which means the availability of trustworthy person to talk confidentially when a problem arose. Also the variables which measured social connectedness showed that the most of them were either a member of a community organization, a society or a club (54.3%) and involved in team work e.g. sport activities (60.9%).

Larger proportion of subjects acknowledged (78.1%) that they were not physically abused during childhood and had a good relationship with parent during childhood (73.3%). When inquiring about exposure to suicidal acts in media, majority (64.7%) revealed that they were exposed to suicidal acts in media.

There were 7 subjects (6.7%) with depression and 6 (5.7%) with alcohol dependence in the study sample. Nine patients (8.6%) were reported to have chronic painful illnesses. Majority (56.1%) had gone through a recent stressful life event. Among all participants, 46.1% and 27.6% had recent (within the last 6 months) dispute with spouse or a close relative respectively. Only 16.1% had a recent meeting with a doctor (within the previous month). Among the participants 15.3% reported a past history of suicidal attempt.

Association between basic demographic characteristics and having recent stressful life events was separately assessed and results are presented in table 4.

Among those who reported a recent stressful life event, 73.3% belonged to nuclear families rather than extended families and it showed a significant

difference (p = 0.097) (level of significance was taken as 10%). Being in a nuclear family was significantly related with exposing into stressful life events. All other variables didn't show significant association with having recent stressful life events.

After assessing the association between basic demographic characteristics and recent dispute in a close relationship, it showed the majority (79.3%) of those who reported recent such an incident were below 25 years and it was a statistically significant difference (p=0.041).

Table 1: Basic demographic characteristics

Characteristic	Subjects (n=105)	
	Frequency	Percentage
Age category (years)		
15 and below	7	6.7%
16 – 25	60	57.2%
26 – 35	20	19.0%
36 – 45	10	9.5%
46 – 55	5	4.8%
65 and above	3	2.9%
Sex		
Female	58	55.2%
Male	47	44.8%
Ethnicity		
Sinhala	102	97.1%
Tamil	1	0.9%
Muslim	2	2.0%
Religion		
Buddhist	101	96.2%
Islamic	2	1.9%
Catholic	2	1.9%
Family income (Rs.)		
Below 10000	46	43.8%
10000 – 30000	58	55.3%
30000 – 60000	0	0%
Above 60000	1	0.9%
Level of education		
No school education	3	2.9%
Up to grade 5	10	9.5%
From grade 6 to 11	75	71.4%
From grade 12 to 13	13	12.3%
Higher education	4	3.9%
Marital status		
Married	53	50.5%
Unmarried	52	49.5%
Employment status		
Currently employed	47	44.8%
Unemployed	58	55.2%
Family type		
Nuclear family	83	79.0%
Extended family	22	21.0%

Socio – cultural characteristics of study sample

Table 2: Distribution of socio – cultural characteristics

Characteristic	Subjects (n=105)	
	Frequency	Percentage
Access to help		
Not available	36	34.3%
Available	69	65.7%
Being a member of any community organization /society /club		
Yes	57	54.3%
No	48	45.7%
Being involved into any team sport		
Yes	64	60.9%
No	41	39.1%
History of physical abuse		
Yes	22	20.9%
No	83	79.1%
Relationship with parents during the childhood		
Not good	28	26.7%
Good	77	73.3%
Exposure to suicidal act in media		
No	37	35.3%
Yes	68	64.7%

Psychological and biological factors of study population

Table 3: Distribution of psychological and biological characteristics

Characteristic	Subjects (n=105)	
	Frequency	Percentage
Presence of Depression		
Yes	7	6.7%
No	98	93.3%
Presence of Alcohol dependence		
Yes	6	5.7%
No	99	94.3%
Presence of a Chronic painful illness		
No	96	91.4%
Yes	9	8.6%
Having recent stressful life event		
Yes	59	56.1%
No	46	43.9%
Recent dispute with spouse		
Yes	24	46.1%
No	28	53.9%
Recent dispute in a close relationship		
Yes	29	27.6%
No	76	72.4%
Recent meeting with a doctor		
Yes	17	16.1%
No	88	83.9%
Family history of suicidal attempt		
Yes	42	40.0%
No	63	60.0%
Past history of suicide attempt		
Yes	16	15.3%
No	89	84.7%

Table 4: Association between basic demographic characteristics and having recent stressful life events

Variable	Having recent stressful life events		Not having recent stressful life events		Significance*
	Frequency	Percentage	Frequency	Percentage	
Age**					
Below 25 years (n=67)	37	6.7%	30	0.9%	$x^2 = 0.278$
Above 26 years (n=38)	23	93.3%	15	99.1%	p = 0.59
Gender					
Male (n=47)	30	50.0%	17	37.8%	$x^2 = 1.55$
Female (n=58)	30	50.0%	28	62.2%	p = 0.21
Family income#					
Below Rs. 10,000 (n= 46)	27	45.0%	19	42.2%	$x^2 = 0.081$
Above Rs. 10,001 (n=59)	33	55.0%	26	57.8%	p = 0.78
Education status##					
Grade 11 and below (n=88)	51	85.0%	37	82.2%	$x^2 = 0.146$
Grade 12 and above (n=17)	9	15.0%	8	178%	p = 0.70
Family type					
Nuclear family (n=83)	44	73.3%	39	86.7%	$x^2 = 2.760$
Extended family (n=22)	16	26.7%	6	13.3%	p = 0.09

* Significance was tested by calculating chi square value (x^2) and p value
** For analysis subjects were divided into 2 age categories
# For analysis subjects were divided into 2 income categories
For analysis subjects were divided into 2 categories of education status

Table 5: Association between basic demographic characteristics and recent dispute in a close relationship

Variable	Having dispute in a close relationship		Not having dispute in a close relationship		Significance*
	Frequency	Percentage	Frequency	Percentage	
Age**					
Below 25 years (n=67)	23	79.3 %	44	57.9%	$x^2 = 4.169$
Above 26 years (n=38)	6	20.7%	32	42.1%	p = 0.041
Gender					
Male (n=47)	15	51.7%	32	42.1%	$x^2 = 0.785$
Female (n=58)	14	48.3%	44	57.9%	p = 0.375
Family income#					
Below 10000 (n= 46)	10	34.4%	36	47.4%	$x^2 = 1.416$
Above 10001(n=59)	19	65.6%	40	52.6%	p = 0.234
Education status##					
Grade II and below (n=88)	26	89.7%	62	81.6%	$x^2 = 1.009$
Grade 12 and above (n=17)	3	10.3%	14	18.4%	p = 0.315
Family type					
Nuclear family (n=83)	21	72.4%	62	81.6%	$x^2 = 1.065$
Extended Family (n=22)	8	27.6%	14	18.4%	p = 0.302

* Significance was tested by calculating chi square value (x^2) and p value
** For analysis subjects were divided into 2 age categories
# For analysis subjects were divided into 2 income categories
For analysis subjects were divided into 2 categories of education status

Discussion

This study was intended to explore the characteristics of people who attempted suicides in Southern Sri Lanka. Basic demographic features and important socio-cultural, psychological and biological characteristics were described separately.

Female dominance in attempted suicides which is known in medical literature (3) was reaffirmed. But bimodal association between age and incidence of attempted suicides which was also shown in local studies (9) was not noted. The ascertainment of outcome totally depended on patient's declaration of the intent. Those who committed the act without intention of killing him/herself may come out with a different reasoning later. Over representation of younger group in the sample can be due to ascertainment bias.

When looked at ethnic and religious composition of Hambantota it is noted that 11% of other ethnic and 10% of other religious population were in the district other than Sinhala Buddhists (10). But incidence of attempted suicides among other ethnic and religious groups were very low. Most of the Muslim community which is the second largest ethnic group in Hambantota live in urban areas and involved in business. So they tend to seek medical care from private sector.

Though being married is noted as a protective factor (11,12) the proportion of married subjects among the sample was 50.5%. Being in a nuclear family increased the likelihood of attempted suicides. But the majority had access to help and socially connected though they are protective factors against suicidal behaviour. Degree of utilization of available support and social networks should be further assessed. Though majority of the sample reported exposure to suicidal acts in media we need to consider that suicide reporting in media is abundant than ever it became more generalized.

Depression and alcohol dependence are two common psychiatric conditions associated with suicidal behavior (3). Reported incidences depend on the accuracy of diagnosis of those conditions by research assistants. But all the subjects with depression in the study sample were females and all with alcohol dependence were males. In Sri Lanka alcohol consumption by women is not socially accepted and a very little number of females take alcohol. Also it is confined to urban areas. Prevalence studies on depression conducted in Sri Lanka show female predominance. But this finding is interesting and it warrants more attentions should be paid on detection and treatment of depression among young females. Both stressful life events and disputes in close relationships are known as precipitating factors for suicidal attempts. Though 56.1% mentioned about recent stressful life events a lesser proportion reported recent disputes with their spouses and in close relationships.

A significant difference was noted in family status of those who reported recent stressful life events. Living in an extended family was noted as a protective factor for exposing into stressful life events, which is a known precipitating factor for suicidal acts. As the family consists of grand parents and siblings opportunity to cope with daily challenges of life is enhanced as more support is available within own family. Therefore strengthening family ties should be promoted.

A significant age difference was noted among those who reported recent disputes in close relationships. Younger patients were subjected to adverse relationship issues more than older subjects. Lack of life skills to maintain a healthy relationship is the apparent reason.

A hospital based sample was taken for this study and hence can lead to selection bias. A community based sample would have given the whole picture though cost and effort arise as barriers. Some factors had shown a contradicting relationship with known literature. An analytical approach using a control group would have elaborated the true association of variables with attempted suicides. Further studies are warranted to establish whether the observed differences are true or not.

Identification of vulnerable groups is of utmost importance in planning public health strategies to prevent attempted suicides. Being below 25 years (precisely 16-25) of age belonging to nuclear families and having had recent stressful life events were noted as vulnerable for attempted suicides. Preventive strategies should be specifically focused on those groups. Utilization of available support, improving personal skills to cope with daily stressors and relationship disputes should be promoted.

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Knowledge, attitudes and practices regarding modern methods of postpartum contraception among postnatal mothers

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ABSTRACT

Introduction: Improving postpartum contraceptive use is an important programmatic strategy to improve the health and well-being of women, newborns and children in a country.

The aim of this study was to assess the knowledge, attitudes and practices regarding postpartum contraception among postnatal mothers.

Method: A cross sectional study on 300 postnatal mothers was carried at Teaching Hospital Mahamodara (THM) Galle. Multiparous mothers were recruited using convenient sampling method. Data collection was done using a validated, self-administered structured questionnaire after obtaining informed consent. Questionnaire was designed to assess on knowledge, attitudes and practices of postpartum contraception.

Result: Level of knowledge regarding postpartum contraception was above average in 15.8% of mothers and below average in 46.6% of them. The majority of them were aware regarding Combined Oral Contraceptives Pills (COCP). Almost 60% of women of Islamic and Hindu religions believed that contraception is not accepted by their religion. Among postnatal mothers, 70.2% had previous practices of postpartum contraception and the commonest method used was COCP. Approximately 76% of mothers gained information regarding postpartum family planning from Public Health Midwives (PHM).

Conclusions: Knowledge on post-partum contraception in general was inadequate. The attitudes and practices on postpartum contraception were influenced by their religion. Short acting contraceptives were popular method of contraception among postnatal mothers. The major information provider was PHM.

Keywords: *Postpartum contraception, Knowledge, Attitudes, Practices*

Introduction

Fertility control with the use of contraceptives is essential to the health and well-being of individuals, family and community. It has been noted that demand fluctuates over the course of reproductive life. Childbirth changes priorities, attitudes and lifestyles of women. Postpartum period is particularly important because adequate birth spacing can improve maternal and infant health (1).

The majority of women resume sexual activity within several weeks of the delivery. The amount of time following delivery that a woman is infertile is highly variable and dependent on multiple factors, including breastfeeding status. Ovulation can occur even if the mother has not resumed menstruation and could happen as early as 25 days postpartum. The probability of ovulation occurring before resumption of menstruation increases over time (2).

Postpartum family planning (PPFP) focuses on the prevention of unintended and closely spaced pregnancies through the first 12 months following childbirth (3).

Globally unmet needs for contraception of women remain high despite of availability of modern safe and effective contraceptive methods. Yet unmet need is constituted a large percentage of total demand in many countries. Unmet need for family planning in Sri Lanka is 15% (4).

Unmet needs could lead to unplanned and unintentional pregnancies which will increase the risk of adverse maternal and neonatal health outcomes. Improving postpartum contraception is an important programmatic strategy to improve the health and well-being of women, newborns, and children (1).

Postpartum contraceptive advice allows women to plan the spacing and number of future children. Short inter-pregnancy intervals have been associated with an increased risk of adverse perinatal outcome; therefore aside from the socioeconomic benefits, delaying future pregnancies may be beneficial in terms of health (5). Postpartum women face uncertainty about timing of return to fecundity. Many women wait to use contraception until menses return, resulting in unintended pregnancies (6).

The choice of a postpartum contraceptive method depends on many factors, including the need for a temporary versus a permanent method, the infant feeding choice and the extent to which informed consent is made prior to delivery. For maximum protection, the non-breastfeeding woman should be protected from the fourth week postpartum, even if that means using a temporary method, such as condoms, until her method of choice is certain (7).

The postnatal period is associated with physio--logical, psychological and social changes, which can influence sexual and reproductive health. Although women may wish to delay or avoid further pregnancy, they may not know how to access contraception or which methods are safe to use, particularly if they are breastfeeding. There may also be difficulties with sexual function and relationships during this time, for which individuals may require information and support (5).

Postpartum contraception is a subject which has been discussed worldwide and it is very important for improvement of their economy. Nearly two-thirds of women in first postpartum year had inadequate contraception in United States of America, for example, nearly 60% of pregnancies conceived at any time are unplanned (23% unintended and 37% mistimed) (8). Surveys from 27 countries indicate that two-thirds of postpartum women had an unmet need for contraception (9).

Ninety five percent of women in low and middle-income countries wish to avoid a pregnancy within next two years, but 70% are not using any method of contraception (10). Common reasons for unmet need for family planning were inconvenience, unsatisfactory services, lack of information, fears about contraceptive side effects and opposition from husbands, relatives or others (11).

In Sri Lanka, the most common reason for illegal termination of pregnancy is youngest child being too young (12). This largely reflects the unmet need among postnatal mothers.

Unmet needs among Sri Lankan women remains approximately 7% among postnatal mothers (13). One of the most common reasons is lack of knowledge regarding different type of contraception methods. The aim of this study was to assess the knowledge, attitudes and practices regarding postpartum contraception among postnatal mothers.

Methods

A descriptive cross sectional study was carried out at Teaching Hospital Mahamodara. Mothers who were in immediate postpartum period were recruited for the study. Postnatal mothers who were not able to read, acutely unwell, suffering from psychiatric illnesses and in intensive care unit were excluded from the study. Three hundred consecutive postnatal mothers were recruited from four postnatal wards during the periods of three months from March to May 2015. Eight postnatal mothers were excluded from the study. Data were collected using a pre-tested self-administered questionnaire that included four sections to evaluate the knowledge, attitudes and practices regarding postpartum contraception among postnatal mothers. It consisted of both open and close ended questions. Section one was designed to obtain basic socio-demographic data. Section two consisted of 33 facts to assess their knowledge about postpartum family planning. Section three was designed with 20 facts to evaluate their attitudes regarding PPFP practices. Section four consisted of four subsections to assess previous practices regarding PPFP. Questions assessing knowledge, attitudes and practices were assigned scores using

predetermined cut-off values. One hundred points were allocated to 33 facts to assess their knowledge about postpartum family planning Postnatal mothers were categorized in to above average (score > 60), average (score 41 to 60) and below average (score < 40) on levels of overall knowledge. Ethical approval was granted by ethical committee of the Faculty of Medicine, University of Ruhuna. Permission for collecting data in hospital was obtained from the Director of the THM.

Participants were fully informed about the purpose of the study and obtained the written informed consent. The privacy and confidentiality of each participant taking part in study was ensured.

Data was entered in to a data base created using Statistical Package for Social Sciences (SPSS) version 20. Data analysis and result presentation were done using mean, p value, standard deviation (SD) and percentages.

Results

Table 1: Basic characteristics of the sample

Socio demographic characteristics		Percentage (n = 292)	
Age	20 -29	32.9%	(96)
	30 -39	61%	(178)
	40 -49	5.8%	(18)
Religion	Buddhist	89%	(260)
	Islam	9.6%	(28)
	Christian	1.4%	(4)
Ethnicity	Sinhala	88.4%	(258)
	Muslim	10.3%	(30)
	Tamil	1.4%	(4)
Level of education	Up to O/L (secondary)	43.8%	(128)
	Up to A/L (secondary)	38%	(111)
	Diploma (high)	4.1%	(12)
	Degree (high)	5.5%	(16)
	Under grade 10 (primary)	8.6%	(25)
Marital status	Married	97.9%	(286)
	unmarried	1.7%	(5)
	Divorced	0.3%	(1)
Income	Not mentioned	10.6%	(31)
	1 - 10,000	8.2%	(24)
	10,001 - 50,000	74%	(216)
	50,001 - 100,000	6.8%	(20)
	>100,000	0.3%	(1)
No of children	2	63.7%	(168)
	3	27.7%	(81)
	4	6.8%	(20)
	≤ 5	1.7%	(5)

According to the age distribution of the sample, majority (61%) was in 30-39 years age group. Almost 90% of mothers were Sinhala Buddhist. Approximately 80% of mothers have had educational level up to ordinary level or above.

Table 2: Overall level of knowledge on postpartum contraception

Level of knowledge	Percentage % (n = 292)
0 - 40 (Below average)	46.6% (136)
41 - 60 (Average)	37.7% (110)
> 60 (Above Average)	15.8% (46)

Approximately half of mothers had poor knowledge regarding postpartum contraception.

Table 3: Willingness of participants to use a contraceptive method after a child birth

Attitudes to wish to use contraceptives	Percentage (n = 292)
Yes	83.6% (244)
No	16.4% (48)

Out of 292 postnatal women more than 80% women wished to use some type of contraceptive after the child birth.

Table 4: Beliefs regarding post-partum contraception

	Yes (%)	No (%)
Delayed till next pregnancy	88.7 % (259)	11.3 % (33)
Not accepted by the religion	8.9 % (26)	91.1 % (266)
Sexual satisfaction is minimal	7.5 % (22)	92.5 % (270)
Fear of side effects	44.2 % (129)	55.8 % (163)
Doubts regarding instructions	5.8 % (17)	94.2 % (257)

Approximately 10% of mothers believed religion is a deciding factor for contraception and 45% mothers has had fear of using a contraceptive method due to its side effects.

A vast majority (70%) used some type of contraceptive method following their previous childbirth (within first six months).

Table 5: Percentages usage of different types of contraceptive methods after previous child birth

	Percentage (%)
Combined Oral contraceptive Pills	40.4% (83)
Injectables (DMPA)	20% (41)
Progesterone containing implant (Judella)	1.46% (3)
IUD (Copper T)	20% (41)
Male condoms	17% (35)
Female condoms	0% (0)
Emergency pills (Levonorgestrel)	0.4% (1)
Emergency IUD	0.4% (1)
Others	0.4% (1)

Almost 40% of mothers had used COCP as a postpartum contraceptive method following previous child birth.

Majority of women (76%) had gained their knowledge regarding PPFP from Family Health Workers. Approximately 10% of them acquired information from both doctors and nurses. Media helped to gather knowledge only in 2.7% of women.

Discussion

The study found that overall knowledge about PPC is generally inadequate among postnatal mothers. Vast majority of postpartum mothers wished to use some kind of contraceptive method to space-out next pregnancy. Two third of mothers have practiced PPFP following last child birth and among them most popular method had been COCP. Family Health Worker had been the major source of their awareness.

Even though we expected high level of knowledge among postnatal mothers, this study revealed inadequate knowledge regarding PPC.

When considering attitudes, vast majority of women (83%) wanted to have some type of contraceptive after child birth. But half of them afraid due to side effects of the contraceptive methods. Significant number of non-Buddhist women believes that contraceptive usage is not accepted by their religion. A 2010 analysis of Demographic and Health Survey data from 17 countries demonstrated that 50 - 88% of women in the first year of postpartum period would like to avoid pregnancy but are not using contraception. Policy efforts for providing family-planning services to postpartum women have primarily focused on the first six weeks after delivery, but the extension of services through the first year postpartum is likely to further improve birth spacing. WHO recommends an interval of 24 months or more before attempting a next pregnancy after a live birth, to reduce the risks of adverse outcomes for mother and child (14). This study shows that the majority of mothers did not know exact timing of using PPC although they were aware regarding contraceptive methods.

In our study 70.2% mothers had previous practice of postpartum contraception and the most popular method had been the COCP. According to Cooper *et al*, approximately one third of women were using a modern contraceptive method in his study (6). In the second decade of the 21st century, the best available figures suggest that the contraceptive pills and the condom remain the most widely used methods in Britain (9). LARC (long acting reversible contraceptives) has high acceptance rate as postpartum contraception in our study population following previous child birth. This is a healthy trend. Kari et al identified across states, that there was a wide range of use of female sterilization and long-acting reversible contraception. Practice of LARC increased $\geq$ 18% per year, while use of injectables and oral contraceptives declined by 2.5-10.6% annually (15). LARC should be encouraged by health care profession as most effective contraceptive method.

Of the mothers those who have had previous practice of postpartum contraception (n = 205), majority (93%) mentioned that the commonest sources information gained was from family health workers. This is probably due to family health worker being their first contact care giver in the community.

However we must encourage other health professionals to counsel mothers at different levels of contact to reduce unmet need of contraception.

Conclusions and recommendations

Knowledge on post-partum contraception in general was inadequate among postnatal mothers. Vast majority of postpartum mothers wished to use some kind of contraceptive method to space-out next pregnancy. Two thirds of mothers have practiced PPFP following last child birth and among them most popular method had been COCP. Family Health Worker had been the major source for their awareness.

We highly encourage health care professionals to counsel eligible couples and postnatal mothers regarding PPFP. This can be facilitated through setting guidelines by policy makers. Modern methods of contraception, specially LARC should be freely available in the family planning clinics for informed choice.

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Epidemiology of attempted suicides in southern Sri Lanka

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ABSTRACT

Suicides are emerging as an important public health issue globally. Suicide rate in Sri Lanka is higher than the global figure and is around 20 per 100,000 population. Knowledge on characteristics of subjects who attempt suicide will help to strengthen prevention programs.

Therefore the aim of this study was to describe characteristics of patients who admitted following attempted suicides. A descriptive cross sectional study was performed using an interviewer administered questionnaire in General hospital Hambantota, which is a tertiary care institute in southern Sri Lanka.

Total sample was consisted of 105 subjects. Mean age was 25.5 (SD 11.93) and 56.2% were females. Except 4 subjects the rest were Sinhala Buddhists. Majority had only secondary education (71.4%), were not employed (55.2%) and belong to nuclear families (79.0%).

Though majority admitted an access to help (65.7%), had good relationship with parents (73.3%) and socially connected, there were 15.3 % with past history of suicidal attempts and 64.7% had exposed to suicidal acts in media. There were 6.7% with depression who were all females and 5.7% with alcohol dependence who were all males. Though 56.1% reported recent stressful life events, only 27.6 % had mentioned about recent disputes in a relationship.

Important outcomes

- Majority of the sample were females, from 16-25 age group, had only secondary education, not employed and belong to nuclear families.
- Larger proportion admitted access to help and socially connected, but majority had reported recent stressful life events.
- There were 6.7% with depression who were all females and 5.7% with alcohol dependence who were all males.

Important limitations

- A hospital based sample was taken for this study and hence led to selection bias and may not give the true picture in community.
- The ascertainment of outcome was totally depended on patient's declaration of the intent. Those who deliberately harmed themselves without a true intention of dying may give a different reasoning later.
- Association with assessed factors could be elaborated better if an analytical approach was taken.

Introduction

Suicides are emerging as an important public health issue worldwide. Every year an estimated number of 900 000 people die by committing suicide. This represents one death every 40 seconds (1). Worldwide incidence of completed suicides is 16 per 100,000 population and it ranks as the 13th leading cause of deaths, globally, according to current World Health Organization (WHO) statistics (2). Ten to twenty times more people attempt suicides than above incidence (3). This adds an immense burden to health care systems worldwide.

Sri Lanka became top ranked in the world by suicide rates in 1995 (47 per 100,000). Although there is a steady and a gradual declining of suicide rates since then, the rate recorded in 2008 was 20.4 per 100,000 of population (4). In 2008, 4120 people succumbed to suicides (3260 males and 860 females) according to the Department of Police data. Though we do not have accurate figures, it is assumed that nearly 40000 people attempt suicides each year. Therefore it is a major socio-economic problem (5) and is high enough to be considered as a top public health issue in the country.

Taking immediate actions to reduce the morbidity and mortality due to suicidal behaviour has become a top priority in WHO agenda. WHO has made a call for a co-ordinated and intensified global action to address this problem. Therefore area specific data is of utmost importance in planning and implementing prevention programs. Evidence based guidance for public health policy making is the key to reduce the burden of suicides.

Attempts to understand and prevent the occurrence of suicidal acts require the assessment of epidemiology of the problem in a given setting (6). Knowledge on characteristics of subjects who attempt suicide in a particular area will help to strengthen prevention programs and yield a better outcome.

Therefore the aim of this study was to describe characteristics of patients who were admitted following deliberate self-harm to a tertiary care institute in southern Sri Lanka.

Methods

Study was conducted in General Hospital Hambantota, a tertiary care institute in the Southern part of the country which caters for a population of nearly one million. A descriptive cross sectional study was performed using an interviewer administered questionnaire as the data collection tool. The data was collected from May to September in the year of 2011.

A person presented with a nonfatal act of self-harm undertaken with conscious self-destructive intent was taken as a case of suicide attempt (7). Ascertainment of attempted suicide was done according to the diagnosis made by the Medical Officer-Mental Health (MO-MH). In routine practice, every deliberate self-harm case is referred to MO - MH who is a postgraduate qualified medical officer in psychiatry who serves under the supervision of a consultant psychiatrist. The interview was performed when the patient was referred for psychiatric assessment by the MO-MH, therefore it was assumed that all the patients selected were physically and mentally capable of providing information. Those who were psychologically or emotionally disturbed or not in a state to undergo the interview were excluded. Eligible subjects were informed about the study and the interview was performed after obtaining written informed consent from the patients. Special emphasis was given to privacy, autonomy, non-maleficence and expected benefits.

Permission from hospital authority was obtained and minimal disruption to the routine patient care was assured. Ethical clearance for the study was obtained from ethics review committee of the Faculty of Medicine, Colombo.

Questionnaire used in the study was a self-constructed one. Selection of variables was done by triangulation of methods. Several text books in psychiatry were scrutinized to identify main aspects of epidemiology of attempted suicides (3,8). Thorough literature survey was done by referring relevant research publications based on the topic. Google scholar and PubMed were explored in finding relevant publications. Finally expert opinion was obtained with the objective of adjusting these variables to local setting. It was pretested with 10 cases in a General Hospital of an adjacent district and further modifications were made.

The questionnaire included background information and items to asses each variable. They were arranged

into a logical order to make the interview more comfortable and facilitate recalling events. Simple language was used. Vague and emotional words were avoided.

The questionnaire was originally developed in English and then translated into Sinhala and Tamil languages. Back translations were done to match with the original English version. Standard diagnostic criteria for research given in ICD 10 classification of mental and behavioural disorders by WHO were used for the assessment of depression and alcohol dependence.

Data were collected by the principal investigator and four research assistants who were former psycho-social workers served under a WHO guided program. To reduce observation bias the research assistants were trained by the principal investigator with special attention on maintaining uniformity. The principal investigator and research assistants were trained by a Consultant Psychiatrist on detecting the two psychiatric conditions assessed in the study using ICD 10 classification of mental and behavioural disorders by WHO.

Patients identified with any of the two psychiatric illnesses assessed were referred to psychiatric unit for appropriate management. Those who needed socio-economic support were referred for pre identified government officers, non-governmental organizations or community based rehabilitation program conducted by the government. Subjects who needed counseling were referred to counseling officers attached to Divisional Secretariat offices in respective areas.

Data analysis was done by SPSS software. Relevant descriptive statistics were calculated and comparisons were done to elicit statistically significant characteristics.

Results

The sample included 105 eligible subjects who agreed to participate.

Basic demographic characteristics of the study sample

Distribution of basic demographic characteristics of the study sample is shown in table 1.

The mean age of study subjects was 25.5 (SD11.9) years and the age range was from 12 to 70. Majority of them (57.2%) belonged to 16 - 25 age group. Females (56.2%) exceeded males in number. Except four subjects the rest of the study population were Sinhala Buddhists. Fifty eight subjects (55.3%) reported a monthly family income of Rs. 10,000/- to 30,000/- while 46 subjects (43.8%) admitted that they had below Rs. 10,000/- monthly family income. Majority of the sample had educated from grade 6 to 11 (71.4%), not employed (55.2%) and belonged to nuclear families (79.0%). Study population consisted of nearly equal portions of married and unmarried subjects (50.5% and 49.5%).

Surprisingly 65.7% of patients admitted that they have an access to help, which means the availability of trustworthy person to talk confidentially when a problem arose. Also the variables which measured social connectedness showed that the most of them were either a member of a community organization, a society or a club (54.3%) and involved in team work e.g. sport activities (60.9%).

Larger proportion of subjects acknowledged (78.1%) that they were not physically abused during childhood and had a good relationship with parent during childhood (73.3%). When inquiring about exposure to suicidal acts in media, majority (64.7%) revealed that they were exposed to suicidal acts in media.

There were 7 subjects (6.7%) with depression and 6 (5.7%) with alcohol dependence in the study sample. Nine patients (8.6%) were reported to have chronic painful illnesses. Majority (56.1%) had gone through a recent stressful life event. Among all participants, 46.1% and 27.6% had recent (within the last 6 months) dispute with spouse or a close relative respectively. Only 16.1% had a recent meeting with a doctor (within the previous month). Among the participants 15.3% reported a past history of suicidal attempt.

Association between basic demographic characteristics and having recent stressful life events was separately assessed and results are presented in table 4.

Among those who reported a recent stressful life event, 73.3% belonged to nuclear families rather than extended families and it showed a significant difference ($p = 0.097$) (level of significance was

taken as 10%). Being in a nuclear family was significantly related with exposing into stressful life events. All other variables didn't show significant association with having recent stressful life events.

After assessing the association between basic demographic characteristics and recent dispute in a close relationship, it showed the majority (79.3%) of those who reported recent such an incident were below 25 years and it was a statistically significant difference (p=0.041).

Table 1: Basic demographic characteristics

Characteristic	Subjects (n=105)	
	Frequency	Percentage
Age category (years)		
15 and below	7	6.7%
16 – 25	60	57.2%
26 – 35	20	19.0%
36 – 45	10	9.5%
46 – 55	5	4.8%
65 and above	3	2.9%
Sex		
Female	58	55.2%
Male	47	44.8%
Ethnicity		
Sinhala	102	97.1%
Tamil	1	0.9%
Muslim	2	2.0%
Religion		
Buddhist	101	96.2%
Islamic	2	1.9%
Catholic	2	1.9%
Family income (Rs.)		
Below 10000	46	43.8%
10000 – 30000	58	55.3%
30000 – 60000	0	0%
Above 60000	1	0.9%
Level of education		
No school education	3	2.9%
Up to grade 5	10	9.5%
From grade 6 to 11	75	71.4%
From grade 12 to 13	13	12.3%
Higher education	4	3.9%
Marital status		
Married	53	50.5%
Unmarried	52	49.5%
Employment status		
Currently employed	47	44.8%
Unemployed	58	55.2%
Family type		
Nuclear family	83	79.0%
Extended family	22	21.0%

Socio – cultural characteristics of study sample

Table 2: Distribution of socio – cultural characteristics

Characteristic	Subjects (n=105)	
	Frequency	Percentage
Access to help		
Not available	36	34.3%
Available	69	65.7%
Being a member of any community organization /society /club		
Yes	57	54.3%
No	48	45.7%
Being involved into any team sport		
Yes	64	60.9%
No	41	39.1%
History of physical abuse		
Yes	22	20.9%
No	83	79.1%
Relationship with parents during the childhood		
Not good	28	26.7%
Good	77	73.3%
Exposure to suicidal act in media		
No	37	35.3%
Yes	68	64.7%

Psychological and biological factors of study population

Table 3: Distribution of psychological and biological characteristics

Characteristic	Subjects (n=105)	
	Frequency	Percentage
Presence of Depression		
Yes	7	6.7%
No	98	93.3%
Presence of Alcohol dependence		
Yes	6	5.7%
No	99	94.3%
Presence of a Chronic painful illness		
No	96	91.4%
Yes	9	8.6%
Having recent stressful life event		
Yes	59	56.1%
No	46	43.9%
Recent dispute with spouse		
Yes	24	46.1%
No	28	53.9%
Recent dispute in a close relationship		
Yes	29	27.6%
No	76	72.4%
Recent meeting with a doctor		
Yes	17	16.1%
No	88	83.9%
Family history of suicidal attempt		
Yes	42	40.0%
No	63	60.0%
Past history of suicide attempt		
Yes	16	15.3%
No	89	84.7%

Table 4: Association between basic demographic characteristics and having recent stressful life events

Variable	Having recent stressful life events		Not having recent stressful life events		Significance*
	Frequency	Percentage	Frequency	Percentage	
Age**					
Below 25 years (n=67)	37	6.7%	30	0.9%	$x^2 = 0.278$
Above 26 years (n=38)	23	93.3%	15	99.1%	p = 0.59
Gender					
Male (n=47)	30	50.0%	17	37.8%	$x^2 = 1.55$
Female (n=58)	30	50.0%	28	62.2%	p = 0.21
Family income#					
Below Rs. 10,000 (n= 46)	27	45.0%	19	42.2%	$x^2 = 0.081$
Above Rs. 10,001 (n=59)	33	55.0%	26	57.8%	p = 0.78
Education status##					
Grade 11 and below (n=88)	51	85.0%	37	82.2%	$x^2 = 0.146$
Grade 12 and above (n=17)	9	15.0%	8	178%	p = 0.70
Family type					
Nuclear family (n=83)	44	73.3%	39	86.7%	$x^2 = 2.760$
Extended family (n=22)	16	26.7%	6	13.3%	p = 0.09

* Significance was tested by calculating chi square value (x^2) and p value
** For analysis subjects were divided into 2 age categories
# For analysis subjects were divided into 2 income categories
For analysis subjects were divided into 2 categories of education status

Table 5: Association between basic demographic characteristics and recent dispute in a close relationship

Variable	Having dispute in a close relationship		Not having dispute in a close relationship		Significance*
	Frequency	Percentage	Frequency	Percentage	
Age**					
Below 25 years (n=67)	23	79.3 %	44	57.9%	$x^2 = 4.169$
Above 26 years (n=38)	6	20.7%	32	42.1%	p = 0.041
Gender					
Male (n=47)	15	51.7%	32	42.1%	$x^2 = 0.785$
Female (n=58)	14	48.3%	44	57.9%	p = 0.375
Family income#					
Below 10000 (n= 46)	10	34.4%	36	47.4%	$x^2 = 1.416$
Above 10001(n=59)	19	65.6%	40	52.6%	p = 0.234
Education status##					
Grade II and below (n=88)	26	89.7%	62	81.6%	$x^2 = 1.009$
Grade 12 and above (n=17)	3	10.3%	14	18.4%	p = 0.315
Family type					
Nuclear family (n=83)	21	72.4%	62	81.6%	$x^2 = 1.065$
Extended Family (n=22)	8	27.6%	14	18.4%	p = 0.302

* Significance was tested by calculating chi square value (x^2) and p value
** For analysis subjects were divided into 2 age categories
# For analysis subjects were divided into 2 income categories
For analysis subjects were divided into 2 categories of education status

Discussion

This study was intended to explore the characteristics of people who attempted suicides in Southern Sri Lanka. Basic demographic features and important socio-cultural, psychological and biological characteristics were described separately.

Female dominance in attempted suicides which is known in medical literature (3) was reaffirmed. But bimodal association between age and incidence of attempted suicides which was also shown in local studies (9) was not noted. The ascertainment of outcome totally depended on patient's declaration of the intent. Those who committed the act without intention of killing him/herself may come out with a different reasoning later. Over representation of younger group in the sample can be due to ascertainment bias.

When looked at ethnic and religious composition of Hambantota it is noted that 11% of other ethnic and 10% of other religious population were in the district other than Sinhala Buddhists (10). But incidence of attempted suicides among other ethnic and religious groups were very low. Most of the Muslim community which is the second largest ethnic group in Hambantota live in urban areas and involved in business. So they tend to seek medical care from private sector.

Though being married is noted as a protective factor (11,12) the proportion of married subjects among the sample was 50.5%. Being in a nuclear family increased the likelihood of attempted suicides. But the majority had access to help and socially connected though they are protective factors against suicidal behaviour. Degree of utilization of available support and social networks should be further assessed. Though majority of the sample reported exposure to suicidal acts in media we need to consider that suicide reporting in media is abundant than ever it became more generalized.

Depression and alcohol dependence are two common psychiatric conditions associated with suicidal behavior (3). Reported incidences depend on the accuracy of diagnosis of those conditions by research assistants. But all the subjects with depression in the study sample were females and all with alcohol dependence were males. In Sri Lanka alcohol consumption by women is not socially accepted and a very little number of females take alcohol. Also it is confined to urban areas. Prevalence studies on depression conducted in Sri Lanka show female predominance. But this finding is interesting and it warrants more attentions should be paid on detection and treatment of depression among young females. Both stressful life events and disputes in close relationships are known as precipitating factors for suicidal attempts. Though 56.1% mentioned about recent stressful life events a lesser proportion reported recent disputes with their spouses and in close relationships.

A significant difference was noted in family status of those who reported recent stressful life events. Living in an extended family was noted as a protective factor for exposing into stressful life events, which is a known precipitating factor for suicidal acts. As the family consists of grand parents and siblings opportunity to cope with daily challenges of life is enhanced as more support is available within own family. Therefore strengthening family ties should be promoted.

A significant age difference was noted among those who reported recent disputes in close relationships. Younger patients were subjected to adverse relationship issues more than older subjects. Lack of life skills to maintain a healthy relationship is the apparent reason.

A hospital based sample was taken for this study and hence can lead to selection bias. A community based sample would have given the whole picture though cost and effort arise as barriers. Some factors had shown a contradicting relationship with known literature. An analytical approach using a control group would have elaborated the true association of variables with attempted suicides. Further studies are warranted to establish whether the observed differences are true or not.

Identification of vulnerable groups is of utmost importance in planning public health strategies to prevent attempted suicides. Being below 25 years (precisely 16-25) of age belonging to nuclear families and having had recent stressful life events were noted as vulnerable for attempted suicides. Preventive strategies should be specifically focused on those groups. Utilization of available support, improving personal skills to cope with daily stressors and relationship disputes should be promoted.

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