Vitamin D supplementation in patients with type 2 diabetes

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Vitamin D, the so called sun-shine vitamin, is a hot topic currently, with number of studies proclaiming its benefits for several non-skeletal illnesses including cancer, cardiovascular diseases, autoimmune and inflammatory diseases, dementia, and diabetes. Conversely, recent studies have been more cautious and questioning its perceived benefits (1).

Along with numerous non-skeletal effects, vitamin D has received its attention on potential pancreatic beta cell function and insulin sensitivity. Historically the role of vitamin D in diabetes was recognized by a seasonal variation in glycemic control reported in patients with diabetes being worse in the winter. This may be due to insufficient vitamin D as a result of reduced sunlight in winter. Additional evidence supported the role of vitamin D in diabetes; presence of vitamin D receptors on pancreatic β cells, expression of 1α hydroxylase activity at pancreatic β cells causing activation of vitamin D, and increased transcription of insulin receptor genes by 1,25(OH)₂D (2). As β-cells in the pancreas are affected via cytokine-induced apoptosis, systemic inflammation has an important role in insulin resistance and cardiovascular events in patients with diabetes. Vitamin D could decrease the effects of systemic inflammation and protect β-cells. Other proposed mechanism where vitamin D affects insulin resistance is through the renin angiotensin aldosterone system (RAAS). Low levels of 1,25(OH)₂D is shown to increase angiotensin II which inhibit the action of insulin in vascular and skeletal muscle tissues, leading to impaired glucose uptake (3).

Large number of cross-sectional studies have generally reported an inverse association between vitamin D status and prevalent hyperglycemia, favouring the biological perspective, where vitamin D have both direct and indirect effects on various mechanisms related to the pathophysiology of type 2 diabetes.

The study by Forouhi et al. reported that the baseline serum vitamin D levels are inversely associated with glucose and insulin levels collected 10 years later(4). Finnish cohort study also showed an inverse association between baseline serum 25(OH) D and 17-year risk of type 2 diabetes, after adjustment for confounders like obesity and low physical activity (5). A post hoc analysis of a trial designed for bone-related outcomes, found that 700 IU/day of Vitamin D3 (combined with calcium) decreased homeostasis model assessment of insulin resistance in participants with impaired glucose tolerance but not in those with normal fasting glucose. Numerous other prospective cohort studies have also demonstrated that higher vitamin D status was associated with reduced risk of type 2 diabetes. The important role of vitamin D in the development of diabetes evidenced by these epidemiological studies prompts clinicians to prescribe more and more vitamin D supplements. Subsequently several researches on the positive preventive effect of vitamin D supplementation were widely carried out. Number of randomized controlled trials study showed that vitamin D supplementation reduced blood glucose, increased insulin sensitivity in diabetes patients, and decreased development of diabetes related complications (6).

On the other hand, there was a lack of correlation between the use of vitamin D and insulin secretion rate or hemoglobin A1c (HbA1c) in patients with diabetes (7). Findings from the investigation by Dr Forouhi and colleagues using data from several studies covering thousands of people of European descent also supported the same. They investigated the link between levels of Vitamin D and risk of developing diabetes by examining the genes that control blood levels of Vitamin D. The researchers did not find any evidence of low Vitamin D causing type 2 diabetes, nor they found a link between the risk of developing type 2 diabetes and the different gene variants that control blood levels of Vitamin D (2).

More recently the findings from the Vitamin D and Type 2 Diabetes (D2d) trial were presented at the American Diabetes Association 2019 scientific sessions by Anastassios Pittas. Among persons at high risk for type 2 diabetes not selected for vitamin D insufficiency, vitamin D3 supplementation at a dose of 4000 IU per day did not result in a significantly lower risk of diabetes than placebo. Study results did not show a statistically
significant benefit for vitamin D in decreasing progression to type 2 diabetes in people who have sufficient levels. However, in a post-hoc analysis of data from the 103 participants with vitamin D deficiency (<12 ng/mL) showed that vitamin D supplementation potentially had a benefit (7).

Other recent systemic analysis also suggested that large dosage, short-term vitamin D supplementation was most likely to yield preferred changes in vitamin D deficient, non-obese groups, Asians, especially Middle Easterners, and patients with optimal glycemic control at baseline (8).

Even though Sri Lanka is a tropical country with sufficient sunlight throughout the year, vitamin D insufficiency is not uncommon among our population. But data on vitamin D status in Sri Lankans are very few. One study showed that vitamin D status among Sri Lankans living in Kandy was reasonable levels (mean 54.2 nmol/l) and a clear seasonal variation with the lowest 25(OH)D levels in August-September and the highest levels in November-December (9). In the national survey, a higher prevalence of calcium deficiency was found among children aged 6–59 months. But there was a marked variation in the prevalence of hypovitaminosis D from 5 to 25% (10). A recent study done in an urban setting in Colombo, Sri Lanka showed 90.2% of cumulative community prevalence of vitamin D deficiency and insufficiency. Prevalence was highest among young and females. Moor ethnicity showed a significant association with vitamin D deficiency. Dysglycaemia was highly prevalent in this population, but it was not associated with vitamin D deficiency(11).

In conclusion, there is a biological plausibility of an important role of vitamin D in type 2 diabetes, and lower vitamin D status and intake are associated with higher risk of incident type 2 diabetes in observational studies; however, the effect of vitamin D supplementation on glycaemic outcomes was inconsistent. Currently there is insufficient data to support the contention that type 2 diabetes can be improved by raising vitamin D concentration. In Sri Lanka confirmation of a potential beneficial effect of vitamin D on type 2 diabetes in our population need to studied in large trials. This should be specifically designed to test the vitamin D status in our population and whether this is a direct contributor to type 2 diabetes pathogenesis. If such an intervention proven effective, this measures could be applied at public health level to decrease diabetes related burden and costs.
1. JoAnn E. Manson, M.D., et al. Vitamin D Supplements and Prevention of Cancer and Cardiovascular Disease. *N Engl J Med* 2019;380:33-44 DOI: 10.1056/NEJMoa1809944

2. Acharya A and Halemani SS: Role of Vitamin D in Diabetes Mellitus. *Int J Pharm Sci Res* 2016; 7(5): 1881-88.doi: 10.13040/IJPSR.0975-8232.7(5).1881-88.

3. Nakashima, Akio et al. “Role of vitamin D in diabetes mellitus and chronic kidney disease.” *World journal of diabetes* vol. 7,5 (2016): 89-100. doi:10.4239/wjd.v7.i5.89.

4. Forouhi NG, Luan J, Cooper A, Boucher BJ, Wareham N. Baseline 25-hydroxy vitamin D is predictive of future glycemic status and insulin resistance: the Medical Research Council Ely prospective study. *Diabetes*2008; 57:2619-25.

5. Mattila C, Kneckt P, Mannisto S, Rissanen H, Laaksonen MA, Montonen J, Reunanen A: Serum 25-hydroxyvitamin D concentration and subsequent risk of type 2 diabetes. *Diabetes Care* 2007;30:2569-70.

6. Rad EY, Djalali M, Koohdani F, et al. The effects of vitamin D supplementation on glucose control and insulin resistance in patients with diabetes type 2: a randomized clinical trial study. *Iranian J Public Health* 2014;43:1651–6.

7. Pittas AG, Harris SS, Stark PC, Dawson-Hughes B. The effects of calcium and vitamin D supplementation on blood glucose and markers of inflammation in nondiabetic adults. *Diabetes Care* 2007;30:980- 6.

8. Xinyi Li ID, Yan Liu, Yingdong Zheng, Peiyu Wang and Yumei Zhang. The Effect of Vitamin D Supplementation on Glycemic Control in Type 2 Diabetes Patients: A Systematic Review and Meta-Analysis. *Nutrients* 2018, 10, 375; doi:10.3390/nu10030375

9. Haakon E Meyer et.al. Vitamin D status in Sri Lankans living in Sri Lanka and Norway. *British Journal Of Nutrition* 99(5):941-4 · May 2008

10. Abeywickrama HM, Koyama Y, Uchiyama M, et al. Micronutrient Status in Sri Lanka: A Review. *Nutrients*. 2018;10(11):1583. Published 2018 Oct 27. doi:10.3390/nu10111583

11. Chandrika J Subasinghe. Et al. Prevalence of vitamin D deficiency/Insufficiency and its metabolic associations in an urban setting in Sri Lanka: Data from Colombo Urban study. *SJDEM*. 2019;9(2):20-28
Forays into the Pathogenesis and Differential Diagnosis of Young Onset Diabetes in India- Insights from Vellore.

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Introduction and Epidemiology

Questions which concern the pathogenesis of diabetes mellitus in the Indian Subcontinent are many, owing to the high prevalence of the disease, the lower age of onset and low body mass index amongst Asian Indians. The reasons as to why these characterize Asian Indian phenotype are innumerable. For one, consanguinity/inbreeding is common in the Indian population enabling certain haplotypes to be expressed with a greater frequency. Changes in lifestyle have occurred which herald the current problem to a significant extent. The foetal origins of diseases-propounded by Barker and colleagues may in part be responsible; considering that the prevalence of low birth weight (LBW) in India is amongst the highest in the world and approaches a figure of 15 to 20% of all live births in some parts of the country.

A study done by our institution and organized in Rural Tripura, the first of its kind in the North East, adjacent to the Bangladesh border demonstrated a prevalence of diabetes of 9%. A similarly unexpectedly high prevalence was seen in rural Arunachal Pradesh on the border with China.

Adolescent Health and the School Going AgeThe focus on academics rather than physical activities occurs from a very early age driven by the Indian school curricular system (SPADES study). Young males aged 14-17 were studied with regards to their glycaemic profile. Impaired fasting glycaemia was present in 20% and nearly 60% of them had an HDL cholesterol of <40 mg/dl; implying that prediabetes had its onset in adolescence. This could be transplotted to full blown diabetes a couple of decades later.

Few years later we analyzed children along with their parents with their anthropometry along with the biochemical analytes. Maternal weight circumference strongly correlated with the waist circumference of the children, indicating an unhealthy phenotype. There were relationships of the maternal waist circumference with the children's waist circumference, lipids and systolic and diastolic blood pressure measurements. If both parents had the metabolic syndrome (MS), the chance of the child having the metabolic syndrome was 6 times greater.

Though this may have been due to learned behaviour transmitted vertically, we undertook a study to see if there was a genetic component. Subjects from the SPADES study were studied for the impact of the FTO single nucleotide polymorphism (SNP) on body habitus. The FTO SNP was had a clear relationship with the waist circumference of children, indicating a partial genetic relationship with the body habitus of these subjects, inducing MS.

Thrifty Genotype HypothesisIn 1961, Neel et al proposed that an individual's adaptation to the environment was dependent on genes selected over a prolonged period (thrifty-genotype). Hales and Barker proposed that suboptimal foetal nutrition, at critical points of time in intrauterine development may cause permanent change in foetal structure, function and metabolism due to foetal programming (thrifty phenotype).

genome–wide association studies (GWAS) from populations in the Vellore birth cohort (VBC), currently 49 years old, had explored genetic variants that modulate birth weight and had identified variants in the ADCY5 (Adenyl Cyclase 5) and CCNL1 (Cyclin L1) locus to have a more robust association with LBW in European populations. The GWAS have also shown that the association with the ponderal index was strong for the near CCNL1 variants, suggesting a greater association of these variants with fat mass than with skeletal growth at birth, there was no association found with adult BMI or the obesity related traits in adulthood.

Interestingly, the birth weight-lowering variants in both Europeans (ADCY5,CDKA L1[Cyclin- dependent kinase 5 regulatory subunit-associated protein like-])and Indians (ADCY5) have displayed significant associations with impaired glucose–insulin homeostasis in adulthood, reinforcing the genetic link between in utero growth, birth weight and type 2 diabetes mellitus (T2DM).

Why do genetic variants fail to influence both ends of the spectrum (birth weight and adult metabolic phenotype) especially in Indians, unlike the Western population? There could be other genetic variants that influence birth weight in Indians and those upstream or downstream exons/mutations responsible for this “negating” effect. Alteration in metabolic capacity in adulthood is resultant to an epigenetic effect in the foetus and infancy. They may have a long-term impact on DNA expression.
Thrifty Phenotype Hypothesis

Insulin resistance (IR) in itself is more prevalent in South Asian youth. These are studies that have been largely done using the HOMA index. There had not been any advanced studies in India to study the insulin sensitivity dynamics in subjects born LBW prior to 2012. Few published studies on indirect calorimetry (IC) prior to this in the metabolic arena in India which were done by us in Fibrocalcific pancreatic diabetes (FCPD) and weightfitters and none in MR resonance spectroscopy in LBW subjects or with a baseline normal metabolic status.

We enrolled 60 LBW and 60 NBW males, born and living in rural environments. LBW adult males were shorter in height and lighter in body weight compared to their NBW counterparts. Moreover, the LBW individuals had a lower lean body mass when compared to NBW counterparts. This difference was present in total body lean mass and extended to the upper and lower limbs of these subjects and was associated with lower bone mineral content in the LBW group.

Interestingly, 8% of the LBW individuals had impaired glucose tolerance, which was not present in the NBW individuals. This was not reflected in the ‘m’ values (measure of insulin sensitivity) - that were obtained from the hyperinsulinemic euglycemic clamp studies (HEC) done on these individuals, who were all associated with a low median BMI (19.5kg/m2) in both LBW and NBW groups. LBW subjects had a marginally significant higher supine resting diastolic blood pressure level when compared to NBW subjects. The measurements in HDL between LBW and NBW subjects were similar.

IC is another tool wherein our group has acquired a lot of experience in using over the years. There was no difference in resting energy expenditure (REE) when measured by IC between the LBW and the NBW group of subjects, nor was there any difference in Glucose or Fat oxidation between the groups. When the data of all 120 subjects were taken as a whole, and the m-value correlated with the IR indices including HOMA-IR, QUICKI, Fasting Insulin levels, Glucose insulin ratio (FGIR), McCauley’s index and Matsuda Index: the strongest correlation was obtained between the m-value and McCauley’s index and Matsuda index. Complex calculations apart, our group showed that the FGIR correlates well with HEC and is superior to HOMA-IR, QUICKI and McCauley’s index, in recent studies. We have evolved a novel equation for calculating fat free mass utilizing bioimpedance measurements.

Dietary intake of protein was significantly lower in LBW subjects when compared to NBW subjects at the time of recruitment. This was associated with a lower proportion of energy being extracted from the intact protein. The parents of LBW subjects were shorter than NBW subjects, suggesting an intergenerational influence on birth weight.

Epigenetic imprinting of LBW maybe profound, however there was uncertainty as to whether the phenotypic disadvantages imbibed in utero would impair the ability to exercise or interfere with improving body composition.

The LBW group had a greater Fat mass (FM)/fat free mass (FFM) reduction when compared to their pre-exercise baseline status and a significant decline in FM/body weight following a 45 minute exercise intervention for 6 weeks on a bicycle. The NBW subjects had a small increase in fat percentage. Moreover, there was a significant reduction in fasting plasma insulin levels in the LBW group, while the reduction was not statistically significant in the NBW. Reductions in insulin secretion, HOMA-IS changes were significant in the LBW and NBWs. Reduction in HOMA-IR was only significant in the NBW.

The same subjects had NMR spectroscopic assessment of micro-quantities of fat in liver and muscle. There was negligible ectopic fat storage in the liver in particular, and to some extent in the muscle, unlike Caucasian subjects. There was no difference in ectopic fat storage between NBW and LBWs. Measurements of IR (HOMA-IR) did not have any relationship with hepatic, intramyocellular or extramyocellular fat content. The only independent predictor of intra-myocellular and extramyocellular fat content was with the total body fat percentage. It would be fair to consider a unifying hypothesis linking the thrifty genotype and phenotype hypothesis, although the exclusive combination would be inadequate to explain an endogenous origin for the increase in young onset diabetes South Asia.

Mendelian Disorders

We examined Mendelian disease as a harbinger of the epidemic of diabetes in India. Maturity Onset Diabetes of the Young (MODY) accounts for up to 2% of patients with diabetes in India. There has also been a trend towards a shift in the age of onset of T2DM to a younger age, ranging from 25 to 34 years. The overlapping clinical feature of MODY with classical polygenic diabetes presents a challenge and requires genetic testing for differentiation.

Genetic testing to identify mutations in a comprehensive panel of ten MODY genes was carried out in 80 subjects of Asian-Indian origin with young onset diabetes. A novel multiplex polymerase chain reaction (PCR) based target enrichment was established, followed by Next Generation Sequencing (NGS) on the Ion Torrent Personal Genome Machine (PGM). All the mutations and rare variants were confirmed by Sanger sequencing. We identified mutations in 11 (19%) of the 56 clinically diagnosed MODY subjects and seven of these mutations were novel. The identified mutations include p.H241Q, p.E59Q, c.-162G>A 5’ UTR in NEUROD1, p.V1691 co-segregating with c.493-4G>A and c.493-20C>T, p.E271K in HNF4A, p.A501S in HNF1A, p.E440X in GCK, p.V177M in PDX1, p.L92F in HNF1B and p.R31L in PAX4 genes. These patients with co-existing NEUROD1-PDX1 mutations showed a marked reduction in glucose induced insulin secretion. None of the subjects who had not met the clinical criteria of MODY were positive for mutations. This was the first report of PDX1, HNF1B, NEUROD1 and PAX4
mutations from India. Multiplex PCR coupled with NGS provides a rapid, cost-effective and accurate method for genetic testing of MODY. When compared to earlier reports, we identified a higher frequency and novel Digenic mutation patterns involving NEUROD1 and PDX1. Subsequent work has shown that unlike the western population where MODY 1, 2 and 3 are the more common forms, MODY 4, 6 and 13 (PDX1, NeuroD1 and ABC8) are commoner.

We asked as to why pregnant young ladies in the early part of the third decade who were non-obese develop gestational/pregestational diabetes (GDM/Pre-GDM). Could they be a subset of individuals with MODY? Young pregnant insulin requiring women were screened for MODY utilizing the same NGS platform. Eighteen percent of subjects who were diagnosed to have GDM/Pre-GDM were MODY positive. Mutations for PDX1, NeuroD1, HNF1a, BLK, INS, ABC8 and GCK were detected in this population. Therefore, MODY may be responsible for at least one-fifth of GDM or Pre-GDM in those with insulin requiring disease.

There are other forms of monogenic diabetes underdiagnosed when utilizing standard Sanger sequencing. For example in Wolfram’s syndrome, there are 8 exons and screening only the 8th exon could miss the appropriate diagnosis in WFS1. The NGS has been utilized for studying the genetic profile of IR in lipodystrophy, an important cause for lean diabetes in the young and requires mega-doses of insulin or respond to pioglitazone; there are milder varieties ranging from Dunnigan Syndrome to the severe Bermedelli-Siep syndrome. NGS is effective in diagnosing H-syndrome.

Sanger sequencing identifies mitochondrial mutations. However, it may depend on the degree of heteroplasmy. In situations of lower heteroplasmy, Sanger sequencing can miss the disorder, and NGS would detect the condition precisely.

Summarizing, NGS is the modality of choice for profiling young onset diabetes, MODY, mitochondrial, Syndromic and neonatal diabetes. At present CMC has a single library preparation handling 40 genes simultaneously and cost-effectively.

**HIV/AIDS Syndrome**

Acquired lipodystrophy in the young could be due to HIV/AIDS, wherein highly active antiretroviral therapy (HAART) precipitates this disorder. Nucleoside reverse transcriptase inhibitors cause selective loss of fat in the face/limbs, and accumulation of abdominal fat. We studied male subjects with HIV aged between 25-50 years of age, comparing the body composition using DXA scans and metabolic parameters of those who had received HAART versus HAART naive, and with those who were HIV- negative. Those subjects, who had received HAART having lipodystrophy, had the highest odds of predicting MS. These patients had a higher proportion of IR, hypertriglyceridemia and lower levels of HDL cholesterol.

**Fibro calcific Pancreatic Diabetes Mellitus**

FCPD is a condition wherein individuals present in the first decade of life with abdominal pain, steatorrhea in the second decade of life and diabetes mellitus in the third decade. The disorder is exclusively present in tropical regions across the world. The pre-diabetic phase characterized by chronic pancreatitis and steatorrhea is called tropical chronic pancreatitis (TCP). The clinical phenotype is well characterized. However, dynamic studies examining insulin secretion, peripheral IR, energy expenditure dynamics, alpha cell function, and incretin output and body composition have not been elucidated. Disease mechanisms are poorly understood.

We undertook several studies to understand these aspects. Using IC we determined the REE in subjects with FCPD. Subjects with FCPD had much higher REE than anticipated. The added factors of poorly controlled diabetes mellitus and malabsorption need correction; this factor in addition to increased REE, indicates that dietary requirements would exceed 2500 to 3500 kcal, since they were underweight at diagnosis. These subjects had a significantly higher intake of fat, fiber, calcium, phosphorus, niacin and higher caloric intake from fat. They had lower carbohydrate and thiamine intake when compared to T1DM subjects.

Studies of body composition showed lowered bone mineral density (BMD) when compared to controls. BMD was inversely related to stool fat excretion and unrelated to vitamin D status. Pancreatic Osteodystrophy was a conglomeration of osteoporosis and Osteomalacia.

We performed HEC and intravenous glucose tolerance tests (IVGTT) along with oral glucose tolerance tests (OGTT) in those subjects with chronic pancreatitis. There was a profound deficiency of insulin secretion, not as severe when compared to matching the insulin secretory defect seen in subjects with T1DM. Patients with TCP had a normal insulin reserve. Studies utilizing the Deuterated glucose measurements (D2G) were performed, which indicated that mild hepatic IR was present (unpublished data).We discovered a paradoxical elevation in glucagon levels in those with FCPD; the levels were somewhere intermediate in comparison to normal controls. Based on this we subsequently proceeded more recently to do OGTT and IVGTT to measure glucagon, pancreatic polypeptide (PP), GLP-1, GIP and Oxyntomodulin levels. The findings were as follows: PP levels were reduced commensurate with depleted islet cell function (p-cell function). The GLP-1 levels were elevated during OGTTs and suppressed during IVGTTs along with the glucagon levels. GIP response was blunted during OGTT and IVGTT as well. Oxyntomodulin was elevated for subjects with FCPD. There is possibly an extra-pancreatic glucagon secretion suggested by the higher level on OGTT with minimal c-peptide and PP response. The source is probably the L-cells, suggested by increased GLP-1, Oxyntomodulin and the correlation between GLP-1 and glucagon. Despite high GLP-1, the incretin effect
is lost, suggesting incretin resistance. K-cell function is probably impaired considering the low GIP response and could be related to hyperglucagonemia (unpublished data).

Ketosis Prone Diabetes (KPD/Flatbush Diabetes) In India, it was thought that patients with diabetic ketoacidosis (DKA) were essentially those presenting with early T1DM and perhaps the older ones presenting with Latent autoimmune diabetes in Adults (LADA). We noticed a number of patients who had diabetes of fulminant onset in youth/early middle age who were GAD antibody negative (GAD-ve). We compared patients who were GAD+ve versus those who were GAD-ve, who had DKA. These two groups of patients were followed up over a period of a year. On following up and monitoring serial c-peptide over a year, it was found that patients who were GAD-ve, a decline in insulin requirements occurred and all subjects were managed entirely on oral antidiabetic agents/nutritional medical therapy. We concluded, for the first time that KPD occurs in Asian Indians.

‘Malnutrition Modulated Diabetes’ (MMD) This condition was characterized in the 1960s and included: diabetes with fasting glucose > 200mg/dl, onset <30 years age, leaness (BMI<18kg/m2), absence of DKA on insulin withdrawal, poor socioeconomic status/childhood history of malnutrition, rural origin and insulin requirement of >60 units a day. There are no radiographic features of FCPD or laboratory evidence of exocrine dysfunction. It is present across lower socioeconomic parts of the world, reeding with improvement in socioeconomic status of the region.

The cause and pathophysiology remain unknown. We performed advanced pancreatic HEC, IC and D2G measurements to quantify hepatic glucose output. All patients had a normal MRI abdomen, GAD-ve and were MODY genetics negative. Patients were found to be insulinopaenic; there was no exaggerated response of glucagon production. Hepatic IR was comparable to those with Type 1 diabetes.

Summary and Unifying Algorithm

In summary, multiple factors are responsible for shift in the phenotype towards the left in India with regards to leaness of body habitus as well as age of these patients with diabetes. In South Asia, one should consider LBW, FCPD, lipodystrophy, mitochondrial diabetes, MODY, KPD, MMD and the HIV-AIDS syndrome on HAART in diabetes in the young. More work is required to identify the cellular pathogenesis to establish the reasons for this propensity.

A proper evaluation involves a detailed history, pedigree charting, proper physical examination for syndromic features, C-peptide levels (fasting and postprandial), imaging of the pancreas, HOMA-IR and DXA where relevant and longitudinal Beta cell monitoring for KPD. Quaternary facilities are required for genetics including NGS, Sanger sequencing and multiple ligation probe dependent amplification for deletions and insertions.

Future directions for research include whole Exome and genome sequencing to elucidate genetic causes for young onset diabetes, fat and muscle biopsies to look for features of peripheral resistance, possible intestinal biopsies with RNA expression and immunostaining for subjects with FCPD, and therapeutic trials of pharmaceutical agents for MODY, FCPD and MMD.
1. Energy Expenditure Studies amongst South Indian Professional Weightlifters. *Ind J Nutr Diet* 2012, 49,433-441. Joseph M, Prema I, Inbakumari M, Jacob KM, Kumar R, Thomas N.

2. Healthcare planning in north-east India: a survey on diabetes awareness, risk factors and health attitudes in a rural community. *J Assoc Physicians India*. 2009; 57:305-9. Lau SL, Debarma R, Thomas N, et al.

3. Awareness and attitude towards diabetes in the rural population of Arunachal Pradesh, North East India. *Ind J Endocrinol Metab* 2012, 16, S83-86. Singh A, Milton PE, Nanniah A, Samuel P, Thomas N.

4. Anthropometric Measurements for the Prediction of the Metabolic Syndrome: A Cross-sectional study on Adolescents and Young adults from Southern India. *Heart Asia* 2011;3: 2-7. Vasan SK, Thomas N et al.

5. Parental determinants of metabolic syndrome among Adolescent Asian Indians: A cross-sectional analysis of parent-offspring trios. *J of Diabetes*. 2015. Baxi R, Vasan SK, Hansdak S, Samuel P, Jeyaseelan V, Geethanjali FS, Murray RR, Venkatesan P, Thomas N.

6. A common variant in the FTO locus is associated with waist-hip ratio in Indian Adolescents. *Pediatr Obes*. 2013;8(3):e45-9. Vasan SK, Fall T, Job V, Gu HF, Ingelsson E, Brismar K, Karpe F, Thomas N.

7. Absence of birth-weight lowering effect of ADCY5 and near CCNL, but association of impaired glucose-insulin homeostasis with ADCY5 in Asian Indians. *PLoS One*. 2011;6(6):e21331. Vasan SK, Neville MJ, Antonisamy B, Samuel P, Fall CH, Geethanjali FS, Thomas N et al.,

8. Born with low birth weight in rural Southern India: what are the metabolic consequences 20 years later? *Eur J Endocrinol*. 2012;166(4):647-55. Thomas N et al.,

9. Indirect Calorimetry: from bench to bedside. *Indian J Endocrinol Metab*. 2017;21(4):594-599. DasGupta R, Ramachandran R, Venkatesan P, Anoop S, Joseph M, Thomas N.

10. Surrogate measures of insulin sensitivity when compared to Euglycemic Hyperinsulinemic Clamp studies in Asian Indian men without Diabetes. *J Diabetes & its Complications*. 2015. Venkatesan P, Tiwari A, Dasgupta R, Carey M, Kehlenbrink S, Wickramanayake A, Jambugulam M, Jeyaseelan I, Ramanathan K, Hawkins M, Thomas N.

11. Bioimpedance analysis with a novel predictive equation - A reliable technique to estimate fat free mass in birth weight based cohorts of Asian Indian males. *Diab Metab Synd: Clin Res Rev* 2019, 13,738-742. Dasgupta R, Anoop S, Samuel P, Kurian ME, Inbakumari M, Finney G, Thomas N.

12. Does being born low birth weight effect the ability to exercise? *Ind J Endocrinol Metab* 2016, 20, 741-743.
Indian J Endocrinol Metab. 2016; 20(6): 741–743. Thomas N et al.Effects of an outdoor bicycle-based intervention in healthy rural Indian men with normal and low birth weight. J Dev Orig Health Dis. 2015;6(1):27-37. Madsen C, Mogensen P, Thomas N et al.

13. Are hepatic and soleus lipid content, assessed by magnetic resonance spectroscopy, associated with low birth weight or insulin resistance in a rural Indian population of healthy young men? Diabet Med. 2016;33(3):365-70. Livingstone RS, Grunnet LG, Thomas N et al.

14. Type 2 diabetes in rural India- new paradigms in its epidemiology and evolution. J Indian Med Assoc. 2009;107(11):785-6, 788, 790. Thomas N et al,

15. Developmental origins of adult metabolic disease: The Indian scenario, driving towards a unified hypothesis. Indian J Endocrinol Metab. 2012;16 (4):493-5. Vasan SK, Thomas N.

16. Molecular Diagnosis of Maturity Onset Diabetes of the Young (MODY) in India. Indian J Endocrinol Metab. 2013;17(3):430-41. Nair V, Arulappan N, Chapla A, Thomas N.

17. Maturity Onset Diabetes of the Young in India. A distinctive mutational pattern identified through targeted next generation sequencing. Clin Endocrinol 2015. Chapla A, Mahesh DM, Asha HS, Varghese D, Varshney M, Vasan S, Venkatesan P, Nair V, Mathai S, Paul T, Thomas N.

18. Comprehensive Maturity Onset Diabetes of the Young (MODY) gene screening in pregnant women with diabetes in India. PLoS One. 2017;12(1):e0168656. Mahesh DM, Chapla A, Asha HS, Varghese D, Varshney M, Paul J, Inbakumari M, Christina F, Varghese RT, Kuruvilla KA, Paul TV, Jose R, Regi A, Lionel J, Jeyaseelan L, Mathew J, Thomas N.

19. Monogenic Diabetes- Diagnostic Conundrums. Int J Diabet Dev Countr 2016. DOI.10.1007/s13410-016-0476-7. Chapla A, Jebasingh FK, Thomas N.

20. Next Generation Sequencing based Genetic Testing for Familial Partial Lipodystrophy. Endocr Practice 2015. Asha HS, Chapla A, Shetty S, Thomas N

21. A novel variant of the AGPAT2 mutation in generalized congenital lipodystrophy, detected by next generation sequencing. Australasian J Med 2016 Shetty S, Chapla A, Kapoor N, Thomas N et al.

22. The H Syndrome: Molecular Diagnosis Using Next Generation Sequencing. AACE Clinical Case Reports 2015. Mahesh DM, Chapla A, Shetty S, Asha HS, Mathew L, George R, Paul TV, Thomas N.

23. HIV lipodystrophy: An objective definition using DXA derived regional fat ratios in a South Asian population. Endocr Pract. 2011:1-32. Asha HS, Seshadri MS, Paul TV, Abraham OC, Rupali P, Thomas N.

24. Emerging concepts in the pathogenesis of diabetes in FCPD (Fibrocalculous Pancreatic Diabetes). J
25. A study on the Resting Energy Expenditure in subjects with Fibro-Calcific Pancreatic. J Diabetes. 2014;6(2):158-63. Behera KK, Joseph M, Sudeep K, Chacko A, Sahoo MK, Mahendri NV, Nair V, Nadig S, Thomas N.

26. Nutritional Intake in Low Body Mass Index (BMI) Males with Type 1 Diabetes and Fibrocalcific Pancreatic Diabetes: What are the Unmet Needs? A Cross-Sectional Study from a South Indian Tertiary Care Hospital. J Clin Diag Res 2017, 11. Joseph M, Dasgupta R, Ramachandran R, Anoop S, Anand V, Devanithi N, Asha HS, Thomas N.

27. Predictors of Osteodystrophy in subjects with chronic non-alcoholic pancreatitis with or without diabetes. Endocr Pract. 2011;17 (6):897-905. Sudeep K, Chacko A, Thomas N et al.

28. Clinical characteristics, Beta-cell dysfunction and treatment outcomes in patients with A-β+ Ketosis-Prone Diabetes (KPD): the first identified cohort amongst Asian Indians. J Diabetes Complications. 2017;31(9):1401-1407. Gupta RD, Ramachandran R, Gangadhara P, Anoop S, Singh SH, Satyaraddi A, Sathyakumar S, Asha HS, Thomas N.

29. Low Body Mass Index Diabetes is Characterized By Impaired Insulin Secretion. Jr Invest Med 2016, 64, 812. Goyal A, Gupta RD, Carey M, Wickramanayake A, Kocherlakota CM, Thomas N et al.

30. Heterogeneity in the aetiology of diabetes mellitus in young adults: A prospective study from North India. Indian J Med Res 2019, 149. Sahoo SK, Zaidi G, Vipin VP, Chapla A, Thomas N, Yu I, Asthana P, Bhatia E.
High prevalence of Diabetes Mellitus in Sri Lankan urban population –
Data from Colombo Urban Study.

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Abstract

Background:
In recent decades, Sri Lanka has experienced rapid urbanization, with approximately 30% of the population currently residing in urban areas. We report the age- and sex-specific prevalence of dysglycaemia in an urban population in Colombo, Sri Lanka.

Methods:
Using a stratified random sampling method, 463 subjects (139 men; 324 women) aged 18 years and above were included. Physical activity was quantified using international physical activity questionnaire (IPAQ). Bio impedence was used to estimate body fat. Insulin sensitivity was estimated using the HOMA calculations. Prevalence was estimated using weighted age standardized calculations. Multiple logistic regression analyses were used to study associations to diabetes and prediabetes.

Results:
There were 124 adults in the 18-40 age group (70% female), 209 adults in the 41-60 age group (73% female) and 130 adults in the > 60 age group (63% female). The overall prevalence of diabetes was 27.6% (95% CI: 23.7-31.4). The prevalence of diabetes in those aged 18-40 was 12.4% (95% CI: 6.4-18.4), 36.1% (95% CI: 29.8-42.4) in those aged 41 – 60 and 48.3% (95% CI: 40.7 – 55.8) in those aged >60. Pre-diabetes was detected in 30.3% (95% CI 25.9-34.8) of the population (with either an HbA1c of 5.7-6.4%, FPG of 110-125 mg/dl or 2 Hr PPG of 140-199 mg/dl). Cumulative prevalence of diabetes and pre-diabetes in the population was 57.9%.

Conclusions:
This urban study demonstrates that along with the changes in the socio-demographic status, the metabolic profile of the Sri Lankan adult has transformed, with a high prevalence of dysglycaemia and obesity.

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Background:

Sri Lanka, a middle-income country in Asia with a population of 20 million, has been experiencing rapid and unplanned urbanization over the recent decades with an estimated 30% of the population now living in urban and suburban areas (1). The urban prevalence of Diabetes, prediabetes and obesity has been rising exponentially over the past three decades. A study in subjects aged over 20 years indicated a population prevalence of dysglycaemia (defined as T2DM or IGT or IFG) of 21.8%, which rose to 30% in urban areas (2). Metabolic syndrome was found in 27.1% of urban adults (3). Physical inactivity, elevated body mass index (BMI) and central obesity along with living in an urban area are thought to be strongly associated with the increased risk of dysglycaemia.

Methods:

The study population consisted of adult males and females who were 18 years and above, whose permanent residence was in the Eastern Kuppiyawaththa local government (Grama-Niladhari) division of the Colombo District. The sample represents an urban population living in Colombo. This local government area was selected for the community cohort as it is the closest to the National Hospital of Sri Lanka, which is the main research center. The study was carried out in 2014/2015.

Sample size

Sample size was calculated using the Lwanga and Lameshow 1991 formula of $n = \frac{z^2 \times p \times (100-p)}{d^2}$. Sample size of 600 was arrived at for expected prevalence of dysglycaemia and obesity of 50%, design effect of 1.2 with a precision of 95% and an anticipated 25% non-response rate using the EPI 6 sample calculation software.

Sampling technique

Stratified simple random sampling was used to select a sample of 463 from the total population of 6473 in the GN area registered in who belonged in the age categories of 18-40 years, 40-60 years and above 60 years. People who are included in the voters list of Colombo district or lives in Colombo district continuously for at least three years were included in the sampling process. In order to ensure the precision of the estimates in the subsample analysis (according to the age groups) the sample was divided among the 3 age categories on a weighted basis that took into account the proportion in the population and the expected prevalence of dysglycaemia.

Using a random number generator, study subjects were randomly allocated into the three strata as follows. In the 18-40 age stratum 210 were selected (35% of total sample) whilst in the 40-60 years stratum 240 were selected (40% of the sample) and in the above 60 year stratum 150 were selected (25% of the sample). The resulting disproportionate sample allocation was accounted for, by the use of weighted analysis. The weights were the inversion of the sampling fractions in the analysis.

Data collection

The participants were recruited at their homes by a team of researchers who provided an invitation letter and information documents. On the day of the screening, informed written consent was taken and data was collected using an interviewer-administered questionnaire administered by trained interviewers, to collect data including socio-demographic data, use of alcohol, smoking, food frequency and physical activity, and detailed medical history on previous diagnoses and treatment. Anthropometric measurements were made (weight, height, waist circumference, total body fat estimation and visceral fat percentage using a bio impedance analyzer- OMRON HBF 516). Blood samples were taken in a nine to twelve hours fasting stage and in non-diabetics 75g anhydrous glucose was given and blood was collected for glucose measurement two hours later. Plasma Glucose (GOD- PAP5 method, Olympus AU 480/680/400 analyser), cholesterol (CHOD-PAP method, Olympus AU 480/680/400 analyser), triglyceride (GPO-PAP method, Olympus AU 480/680/400 analyser), glycosylated hemoglobin (HPLC method, Bio-Rad Variant II Turbo analyser), serum insulin (Chemiluminescent enzyme immunoassay, Immulite 1000 analyser), corrected calcium (Arzenso III method, Olympus AU 480/680/400 analyser) and 25-OH Vitamin D level (Direct Chemiluminescence method, Advia centaur analyser) were measured in the blood samples. Once the serum insulin levels were analyzed, insulin resistance and beta cell function were calculated using the HOMA calculator (4). Diabetes mellitus was diagnosed based on the ADA/WHO criteria. This required either a documented prior diagnosis of diabetes or a value above the diagnostic threshold on biochemical testing. The cutoffs included a FPG above 126mg/dl, 2 Hour PPG above 200 mg/dl, or HBA1C above 6.5%. Pre-diabetes was diagnosed with any of the following values: FPG of 110-125 mg/dl or 2 Hour PPG of 140-199 or an A1C of % 5.7-6.4%.
Statistical analysis:

Data analysis was performed in the R programming language version 3.2.2 (5). Community based prevalence rates and means with 95% confidence intervals for the urban study population and for different strata including age and gender were calculated considering the stratified sampling methodology using the “Survey” package in the R programming language (5). Age adjusted prevalence rates were calculated based on direct standardization method using the World Health Organization world standard population. Descriptive data analysis was carried out to describe study population characteristics. Exploratory data analysis was done to identify the risk factors associated with diabetes mellitus. Exposure variables studied were age, gender, ethnicity, education level, smoking habits, alcohol consuming habits, family history of diabetes, hypertension and hyperlipidemia, past medical history of diabetes, hypertension and hyperlipidemia, weight, height, body mass index (BMI), waist circumference, neck circumference, body fat percentage, visceral fat percentage, physical activity which was quantified as metabolic equivalent of tasks (METS minutes per week) based on International Physical Activity questionnaire (6), food habits based on one week food recall in the Food Frequency Questionnaire and vitamin D levels. Initially, each study variable was screened with Pearson’s Chi-square test and simple logistic regression, and the variables significant at P =0.2 level were subsequently used for multiple variable analysis. Subsequently, multiple logistic regression was carried out to investigate the factors associated with diabetes status and stepwise selection method was adopted to select significant variables. Ethnicity consisted of 4 categories (i.e. Sinhalese, Tamils, Moors and other), the “other” ethnicity had only 4 individuals and this group was not considered in the analysis. P value of 0.05 was considered as significant.

Ethical Issues:

Ethical approval was obtained from the Ethical Review committee of the Faculty of Medicine, University of Colombo. Documents were encoded to avoid any identifying character and measurements were taken to ensure confidentiality.

Results:

A total of 463 subjects gave informed consent and completed the screening. Most of the respondents were females (69%). There were 124 subjects in the 18-40 age group and 70% of these were females. There were 209 subjects in the 41-60 age group and 73% of this stratum were females. In the over 60-year age stratum there were 130 subjects and 63% were females. The response rate in each of the above strata was 59%, 87%, and 87% with an overall response rate of 77.2%. Table 1 summarizes the basic characteristics of this study population.

This study population’s mean Body mass index was 25.2 kg/m2 (SD 4.8), mean waist circumference was 87.0 cm (SD 13.0), mean neck circumference was 34.8 cm (SD 3.7), mean total body fat percentage estimated with bio impedance analysis was 34.3% (SD 8.3) and the estimated visceral fat was 9.2% (SD 5.0). Body mass index was categorized according to global criteria and the recommended Asian and South Asian criteria (7,8). 68.2% of the women and 59.1% of the men were overweight or obese based on South Asian criteria. The BMI categorization and distribution are tabulated in Table 2. Community prevalence for abdominal obesity was 58.1% based on International Diabetes Federation cut-off values on waist circumference for determining abdominal obesity in South Asians (WC – male >= 90cm, female 80cm) (9).

Prevalence of DM and Pre-diabetes

Estimated community mean fasting plasma glucose level was 101.3 mg/dL (95% CI: 97.6 – 105.3), HBA1C level was 6.3% (6.1 – 6.4) and 2 hour PPG in non-diabetic individuals was 124 (119.7 – 129.2). It is notable that these are relatively high and the mean HBA1C is in the prediabetes range. These are tabulated in table 3. Family history of Diabetes Mellitus in at least one first degree relative was reported in 43.2% (95% CI 38.4-48.2) of the study population and 16.9% (95% CI 13.7-20.1) had previously been diagnosed to have diabetes mellitus.

Estimated community prevalence of diabetes mellitus was 27.6% (95% CI 23.7-31.4) and prediabetes was seen in 30.3% (95% CI 25.9-34.8) of the population. Age adjusted community prevalence of diabetes was 27.1% and prediabetes was 30.1%. The community prevalence of dysglycemia was 57.9%; this was seen in 36.2% in the 18-40 age stratum, in 70.7% in the 41-60 stratum, and in 83.5% in the over 60 stratum. Thus, population prevalence with normoglycaemia declined from 63.8% in the 18-40 age stratum to 29.3% in the 41-60 age stratum to 16.5% in the over 60 age stratum (Table 4).

Among ethnic groups, Moors had the highest prevalence of Diabetes Mellitus of 36.1% (95% CI 25.5-46.7) followed by Sinhalaese (30%, 95% CI 22.4-31.6) and Tamils (19.4, 95% CI 8.8-29.9). Those with the lowest educational background had the highest prevalence of diabetes (39.1% (95% CI 28.2-50). Current tobacco smokers had higher prevalence of diabetes mellitus (50.1%, 95% CI 33.4-66.7) compared to those who never smoked (25.2%, 95% CI 21.4-29.1). Ex consumers of alcohol had the highest prevalence of diabetes mellitus (52.9%, 95% CI 31-74.8) compared to non-consumers of alcohol or current consumers (Table 5).
Hundred and two subjects (16.9%) had prior diagnosis of diabetes mellitus. We further analyzed this subgroup who were already diagnosed to have diabetes mellitus and estimated level of control. Mean HbA1C in those who had prior diagnosis of diabetes mellitus was 8.3% (95% CI: 7.9 – 8.8) and mean HOMA β was 47.6 (95% CI: 33.3-61.8) indicating declining insulin reserve. HBA1C less than 7% was found in 29.3% (19.7 – 38.9%), HBA1C between 7% and 8% in 29.5% (20.1 - 38.8%), and HBA1C above 8% was found in 41.2% (31.0% – 51.5%). In the population previously diagnosed with diabetes, blood pressure more than 130/90 mmHg was detected in 33.4% (95% CI: 24.2 –42.6), LDL cholesterol above 100mg/dl was found in 58.1 (48.9 - 69.0), and triglyceride above 150mg/dl was found in 41.3% (31.4 – 51.8).

**Explorative analysis**

Multiple variable analysis showed increasing age, family history of diabetes, preexisting hypertension, increasing BMI, increasing neck circumference, higher frequency of consuming egg yolk and whole grain and less sweet consumption had significant associations with diabetes. Lifestyle factors such as level of physical activity or amount of sitting time recorded with IPAQ did not demonstrate any significant association with the presence of diabetes in the analysis (Table 6).

**Discussion**

The incidence and the prevalence of diabetes mellitus is a rapidly rising in Sri Lanka as well as globally. Urban population is at a higher risk due to multiple predisposing factors. This study was done to ascertain the true urban prevalence of diabetes mellitus as increasing numbers of diabetic patients living in urban areas are encountered in clinical settings. There was an alarmingly high prevalence of dysglycemia in the urban population studied. This is of enormous clinical and economic significance as even the younger population in the 18-40 age stratum had a prevalence of diabetes mellitus of 12.4% and prediabetes of 24.8% with potential to conversion to diabetes in the near future. The high prevalence that has been shown in our study is higher than urban prevalence of 18% according to Katulanda et al nine years ago [2]. It is possible that the prevalence has actually increased, however this study used HBA1C in addition to the FPG and 2 Hour PPG used in the previous study and this may explain part of the increase in prevalence. A previous Colombo suburban study that used all three biochemical parameters reported a prevalence of 20% (10).

Among the factors explored in this study; increasing age, increasing BMI, increasing neck circumference and presence of hypertension as well a family history of diabetes mellitus in a first degree relative, high frequency of consuming egg yolk, whole grain and less sweet consumption are significant in multiple regression analysis. Even though less whole grain consumption and more sweet consumption are believed to be associated with diabetes, our results showed the inverse. This need to be carefully interpreted as already diagnosed diabetics tend to eat less sweets and more whole grain as a diabetes control measure. We also found that Vitamin D level was not significantly associated with diabetes mellitus and the results will be discussed in detail in another article. Several key causative factors have not been explored in this study; they include genetic and epigenetic factors as well as foetomaternal environment, childhood feeding and childhood exercise.

**Conclusions**

We have detected the highest reported prevalence of diabetes mellitus in the South Asian region and these prevalence rates are alarming. The existing pool of patients with diabetes who are likely to develop significant morbidity over time is a major policy and health planning concern. The presence of a large number of individuals who have prediabetes and can develop diabetes in the future should prompt urgent nationwide interventions as well as personalized interventions such as dietary and exercise counselling. We have previously reviewed possible public health interventions to prevent diabetes and other non-communicable diseases in South Asia (11). In light of the current findings, these interventions may need to be targeted more towards the above high risk groups in the urban population.

**List of abbreviations**

- BMI - Body mass index
- FPG - Fasting Plasma Glucose,
- 2 Hour PPG - Post prandial Glucose 2 hours after 75 g glucose
- LDL - Low-density lipoprotein cholesterol,
- HOMA β - Homeostasis Model Assessment estimate of steady state beta cell function
- HDL - High-density lipoprotein cholesterol
- TG - Triglycerides
- HBA1c - Haemoglobin A1c
- TSH - Thyroid stimulating hormone,
- WHO - World health organization
- WC - Waist circumference

**Competing interests:**

Authors declare no conflict of interest

**Ethics approval and consent to participate**

Ethical approval was obtained from the Ethical Review committee of the Faculty of Medicine, University of Colombo. All participants who enrolled in the study signed an informed consent form.
Consent for publication

Not applicable.

Availability of data and materials

The data analyzed in this paper can be made available to researchers. Requests for access to the dataset used in this paper should be directed to the corresponding author.

Competing interests

None of the authors have any financial or non-financial competing interests to disclose.

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Authors’ contributions

NPS and KG designed the study and were involved in data collection. NPS, DSE, IR and KG were involved in statistical analysis, interpretation of data and drafting the manuscript. All authors read and approved the final manuscript.

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None

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References:

1. Department of Census and Statistics Census Report - 2012, Ministry of Finance and Planning, Sri Lanka; 2012
2. Katulanda P, Constantine GR, Mahesh JG, Sheriff R, Seneviratne RDA, Wijeratne S, Wijesuriya M, McCarthy MI, Adler AI, Matthews DR: Prevalence and projections of diabetes and pre-diabetes in adults in Sri Lanka–Sri Lanka Diabetes. Diabetic Medicine 2008;25:1062-9.
3. Katulanda P, Ranasinghe P, Jayawardana R, Sheriff R, Matthews DR. Metabolic syndrome among Sri Lankan adults: prevalence, patterns and correlates. Diabetol Metab Syndr.2012 May 31;4(1):24.
4. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985). "Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man.". Diabetologia 28 (7): 412–19
5. R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/
6. Craig, C. L, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE et al. (2003). "International physical activity questionnaire: 12-country reliability and validity." Med Sci Sports Exerc 35: 1381-95
7. World Health Organization. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet. 2004; 363:157–63
8. Somasundaram N, Rajaratnam H, Wijeyarathne C et al. Clinical guidelines: The Endocrine Society of Sri Lanka: Management of obesity. Sri Lanka Journal of Diabetes, Endocrinology and Metabolism 2014; 1: 55-70.
9. Alberti KGM.M, Zimmer PZ, Shaw JE. The metabolic syndrome—a new world-wide definition from the International Diabetes Federation consensus. Lancet. 2005; 366:1059-62.
10. Pinidiyapathirage MJ, Kasturiratne A, Ranawaka UK, et al. The burden of diabetes mellitus and impaired fasting glucose in an urban population of Sri Lanka. Diabetic Medicine. 2013;30(3):326-332.
11. Somasundaram NP, Kalupahana NS. Population-based dietary approaches for the prevention of noncommunicable diseases. WHO South-East Asia J Public Health 2016; 5(1): 22–26
### Table 1. Study group characteristics.

|                  | Both sexes (N=463) | Males (N=143) | Females (N=320) |
|------------------|---------------------|----------------|-----------------|
| Mean age (SD) years | 50.4 (14.8)         | 50.9 (15.8)    | 50.2 (14.3)     |
| Mean height (SD) cm | 153.3 (9.1)         | 165.0 (7.5)    | 152.4 (6.8)     |
| Mean weight (SD) kg | 61.5 (12.6)         | 65.7 (13.0)    | 59.7 (12.0)     |
| Mean BMI (SD) kg/m2 | 25.2 (4.8)          | 24.1 (4.4)     | 25.7 (4.8)      |
| Mean waist circumference cm | 87.0 (13.0) | 87.5 (12.9) | 86.8 (13.0) |
| Mean neck circumference cm | 34.8 (3.7) | 36.3 (3.3) | 33.0 (3.4) |
| Mean body fat % | 34.3 (8.3)          | 26.5 (7.1)     | 37.8 (6.1)      |
| Mean visceral fat % | 9.2 (5.0)           | 9.8 (5.5)      | 8.9 (4.7)       |
| Ethnicity        |                     |                |                 |
| Sinhala          | 320 (69.1%)         | 108 (75.5%)    | 212 (66.2%)     |
| Tamil            | 56 (12.1%)          | 12 (8.4%)      | 44 (13.8%)      |
| Moor             | 83 (17.9%)          | 23 (16.1%)     | 60 (18.8%)      |
| Other            | 4 (0.8%)            | -              | 4 (1.2%)        |
| Education        |                     |                |                 |
| Below Grade 5    | 77 (16.7%)          | 10 (7.1%)      | 67 (20.6%)      |
| Up to Ordinary Level | 240 (51.9%) | 77 (53.8%) | 163 (51.1%) |
| Up to Advanced Level | 127 (27.5%) | 47 (32.9%) | 80 (25.1%) |
| Above Advanced Level | 18 (3.9%)  | 9 (6.2%)      | 9 (2.8%)        |
| Tobacco smoking  |                     |                |                 |
| No               | 396 (85.6%)         | 83 (58.0%)     | 313 (97.9%)     |

### Table 2. Prevalence (%) of underweight, normal weight, over weight and obesity according to Global, Asian and South Asian BMI categories

|                  | Underweight | Normal | Overweight | Obesity |
|------------------|-------------|--------|------------|---------|
| Global BMI cut offs | 7.7 (4.9-10.4) | 39.6 (34.8-44.4) | 37.0 (32.2-41.8) | 15.8 (12.3-19.3) |
| Asian BMI cut offs | 7.7 (4.9-10.4) | 26.8 (22.4-31.2) | 34.3 (29.6-39.0) | 31.2 (26.7-35.8) |
| South Asian BMI cut offs | 7.6 (4.9 – 10) | 26.8 (22.4-31.2) | 12.7 (9.6-15.9) | 52.8 (47.8 – 57.7) |
| Measure                      | Both sexes | Males       | Females    |
|------------------------------|------------|-------------|------------|
| FBS                          | 101.3 (97.6 – 105.3) | 106.2 (97.1 – 115.4) | 99.6 (95.4 – 103.7) |
| OGTT 2 hr*                   | 124.4 (119.7 – 129.2) | 122.9 (113.9 – 132.0) | 125.1 (119.4 – 130.7) |
| HBA1C                        | 6.3 (6.1 – 6.4)   | 6.4 (6.1 – 6.7)   | 6.2 (6.0 – 6.4)   |
| Plasma Insulin               | 5.9 (5.2 – 6.7)   | 5.8 (4.9 – 6.7)   | 6.0 (5.0 – 7.1)   |
| HOMA IR                      | 1.6 (1.3 – 1.8)   | 1.5 (1.3 – 1.8)   | 1.6 (1.2 – 1.9)   |
| HOMA β                       | 89.7 (77.3 – 102.0) | 83.0 (68.9 – 97.2) | 92.7 (75.9 – 109.4) |
| Family history of Diabetes   | 43.2 (38.4 – 48.2) | 45.6 (36.7 – 54.5) | 42.2 (36.3 – 48.1) |
| Past medical history of Diabetes | 16.9 (13.7 – 20.1) | 18.1 (11.9 – 24.2) | 16.4 (12.6 – 20.2) |

*In non-diabetic individuals

| Measure                      | Community | 18-40 | 41-60 | 60 + |
|------------------------------|-----------|-------|-------|------|
| Normal                       | 42.1 (37.5 – 46.8) | 63.8 (54.1 – 71.6) | 29.3 (23.3 – 35.3) | 16.6 (10.9 – 22.2) |
| Prediabetes                  | 30.3 (25.9 – 34.8) | 24.8 (16.9 – 32.6) | 34.6 (28.4 – 40.9) | 35.2 (27.9 – 42.4) |
| Diabetes                     | 27.6 (23.7 – 31.4) | 12.4 (6.4 – 18.4)  | 36.1 (29.8 – 42.4) | 48.3 (40.7 – 55.8) |

| Measure                      | Community | 18-40 | 41-60 | 60 + |
|------------------------------|-----------|-------|-------|------|
| Normal                       | 40.3 (31.4 – 49.2) | 61.1 (45.4 – 76.8) | 25.4 (14.7 – 36.1) | 16.7 (6.8 – 26.5) |
| Prediabetes                  | 27.2 (19.5 – 34.8) | 19.4 (6.7 – 32.2)  | 33.9 (22.3 – 45.6) | 33.3 (20.9 – 45.7) |
| Diabetes                     | 32.5 (24.6 – 40.4) | 19.4 (6.7 – 32.2)  | 40.7 (28.6 – 52.7) | 50.0 (36.8 – 63.2) |

| Measure                      | Community | 18-40 | 41-60 | 60 + |
|------------------------------|-----------|-------|-------|------|
| Normal                       | 43.0 (37.2 – 48.7) | 63.6 (53.1 – 74.2) | 30.8 (23.6 – 38.0) | 16.5 (9.6 – 23.4) |
| Prediabetes                  | 31.8 (26.3 – 37.2) | 27.3 (17.5 – 37.1) | 34.9 (27.5 – 42.4) | 36.1 (27.2 – 45.0) |
| Diabetes                     | 25.3 (20.8 – 29.7) | 9.1 (2.8 – 15.4)   | 34.2 (26.8 – 41.6) | 47.4 (38.2 – 56.7) |
### Table 5. Prediabetes, diabetes based on categories of education, ethnicity, tobacco, and alcohol

| Ethnicity          | Normal          | Prediabetes     | Diabetes        |
|--------------------|-----------------|-----------------|-----------------|
| Sinhala            | 41.0 (35.2 – 46.8) | 32.0 (26.5 – 37.5) | 30.0 (22.4 – 31.6) |
| Tamil              | 60.0 (46.7 – 73.2) | 20.7 (10.4 – 30.9) | 19.4 (8.8 – 29.9) |
| Moor               | 32.3 (20.9 – 43.7) | 31.6 (21.3 – 41.9) | 36.1 (25.5 – 46.7) |

| Education          | Normal          | Prediabetes     | Diabetes        |
|--------------------|-----------------|-----------------|-----------------|
| Below Grade 5      | 26.5 (23.6 – 45.2) | 34.4 (15.2 – 37.7) | 39.1 (28.2 – 50.0) |
| Upto Ordinary level| 41.2 (25.5 – 37.9) | 31.7 (34.5 – 47.9) | 27.1 (21.5 – 32.6) |
| Upto advanced level| 46.5 (20.4 – 37.4) | 28.9 (37.1 – 55.9) | 24.6 (17.4 – 31.9) |
| Above advanced level| 69.5 (0.0 – 26.6) | 10.8 (47.3 – 91.7) | 19.7 (1.6 – 37.8) |

| Tobacco smoking    | Normal          | Prediabetes     | Diabetes        |
|--------------------|-----------------|-----------------|-----------------|
| Never              | 44.0 (39.0 – 49.0) | 30.7 (25.9 – 35.5) | 25.2 (21.4 – 29.1) |
| Current smokers    | 33.0 (17.0 – 49.0) | 16.9 (6.4 – 27.3) | 50.1 (33.4 – 66.7) |
| Ex-smokers         | 25.7 (6.3 – 45.1) | 45.5 (25.1 – 65.9) | 28.8 (10.9 – 46.6) |

| Alcohol consumers  | Normal          | Prediabetes     | Diabetes        |
|--------------------|-----------------|-----------------|-----------------|
| Never              | 43.9 (38.6 – 49.1) | 30.2 (25.3 – 35.2) | 25.9 (21.7 – 30.0) |
| Current consumers  | 38.6 (25.9 – 51.3) | 31.7 (20.0 – 43.5) | 29.7 (19.3 – 40.0) |
| Ex-consumers       | 19.5 (3.7 – 35.3) | 27.6 (10.0 – 45.2) | 52.9 (31.0 – 74.8) |

### Table 6. Significant variables at Multiple variable analysis for the presence of diabetes mellitus

|                      | Estimate | Std. Error | z value | Pr(>|z|) |
|----------------------|----------|------------|---------|---------|
| Intercept            | -9.04    | 1.479      | -6.11   | <0.001  |
| Age                  | 0.04     | 0.009      | 3.54    | <0.001  |
| Hypertension         | 0.92     | 0.260      | 3.54    | <0.001  |
| Family History of Diabetes mellitus | 0.96     | 0.236      | 4.08    | <0.001  |

|                      | Estimate | Std. Error | z value | Pr(>|z|) |
|----------------------|----------|------------|---------|---------|
| BMI                  | 0.06     | 0.029      | 1.98    | 0.048   |
| Neck circumference   | 0.10     | 0.039      | 2.46    | 0.014   |
| Egg yolk             | 0.28     | 0.108      | 2.62    | 0.008   |
| Whole grain          | 0.19     | 0.077      | 2.43    | 0.015   |
| Sweets               | -0.27    | 0.111      | -2.46   | 0.014   |
Prevalence of vitamin D deficiency/Insufficiency and its metabolic associations in an urban setting in Sri Lanka: Data from Colombo Urban study

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Abstract

Introduction:
Vitamin D deficiency is a commonly prevalent, but less attended problem in Asia. Vitamin D status has many metabolic associations. We designed this community-based study to describe the prevalence of vitamin D deficiency/insufficiency and its metabolic associations in Sri Lankan population.

Methods:
A representative sample aged 18 years and above was included. Demographic, anthropometric, and social details were recorded using a standard proforma. Blood analysis was done for vitamin D status, and other metabolic parameters. Prevalence was estimated using weighted age standardized calculations. Multiple logistic regression analyses were used to study associations to vitamin D status.

Results:
Cumulative community prevalence of Vitamin D deficiency and insufficiency was 90.2%. Prevalence was highest among young and females. Obese had significantly lower vitamin D levels. According to the linear regression, Moors showed a significantly lower Vitamin D levels compared to Sinhalese while Triglyceride levels showed an inverse association with Vitamin D levels. Dysglycaemia was not associated with Vitamin D deficiency.

Discussion:
Very high prevalence of Vitamin D problem was anticipated on clinical grounds and this is comparable with regional data. High prevalence among young needs early attention to avoid future poor bone health outcomes. Moor ethnicity shows high rates due to many known factors. Obesity is an emerging health problem in the country and co existent Vitamin D deficiency would increase its burden.

Conclusion:
Vitamin D deficiency and insufficiency and other metabolic problems are highly prevalent in this population. Causative factors and consequences of this problem should be further researched to plan strategies to replete the Vitamin D and prevent this problem.

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Introduction

Vitamin D deficiency is pandemic and widespread across the world irrespective of age, gender, race and geography, yet it is one of the most under-diagnosed and under-treated nutritional deficiency. Cut off values for vitamin D deficiency has been debated long, but lately, the Institute of Medicine (IOM) has defined Vitamin D deficiency as a 25(OH)D of less than 20 ng/ml and Vitamin D insufficiency as a 25(OH)D of 21–29 ng/ml (1,2).

Vitamin D is unique among hormones because it can be photosynthesized in skin on exposure to Ultraviolet B rays, and thus adequate sun exposure alone ought to suffice for vitamin D sufficiency. However, studies from South Asia over last decade have shown that hypovitaminosis D is highly prevalent (70-100%) across the region despite plentiful sunshine in the area (1-5). Natural dark skin and diversified socio-cultural, religious and economic factors of the region are thought to be contributing to this problem. People with a dark skin tone have natural sun protection and require at least three to five times longer exposure to make the same amount of vitamin D as a person with a white skin tone (6,7). Rapid unplanned urbanization in the South Asian region associated with air pollution and lack of outdoor activities has further compounded this problem (1).

Sri Lankan data on vitamin D status is limited, but according to two community based studies, prevalence of vitamin D deficiency (defined as 25(OH)D less than 25nmol/l) was 6.5% among community dwelling Ethnic Tamil women from central hills and 40.5% in a cohort of women from Southern coastal area (8,9). Despite absence of strong data, very high prevalence of vitamin D deficiency/insufficiency is anticipated due to increased numbers of individuals with this problem encountered daily in the Sri Lankan clinical setting.

Many cross sectional, observational studies have identified several important metabolic associations of vitamin D deficiency, even though their cause-effect relationship is not yet well established. Association between obesity and vitamin D deficiency is well known, although the exact explanation for this is unknown. It has been postulated that obese individuals may avoid sun exposure and consume micronutrient deficient diet. Alternatively, their production of active vitamin D metabolites is enhanced, which in turn exerts negative feedback on vitamin D synthesis in liver. In addition, it is suggested that the metabolic clearance of vitamin D may increase in obesity, possibly with enhanced uptake by adipose tissue (10-12).

Several meta-analyses of observational studies have showed inverse relation of 25(OH)D levels with insulin resistance, hyperglycemia metabolic syndrome and cardiovascular morbidity. Evidence has shown that, Vitamin D receptors are present in pancreatic beta cells and peripheral target organs (skeletal muscles), and thus play a important role in regulating insulin secretion, as well insulin sensitivity. However, interventional studies and randomized clinical trials have shown conflicting results on the effects of vitamin D repletion on dysglycaemia and metabolic profile (13-16).

Therefore, we designed this community based cross-sectional study in an urban setting in Sri Lanka to describe the community-based prevalence of vitamin D deficiency and insufficiency in this population. We further analyzed our data to revisit the known metabolic associations to the vitamin D status in our population.

Methodology

Subjects and Sampling: A community based descriptive cross-sectional study was carried out in the Eastern Kuppiyawaththa local government (Grama-Niladhari) division of the Colombo District, which was selected as it is the closest to the main research center, National Hospital of Sri Lanka during 2014/2015. This Vitamin D prevalence study was carried out as a major component of Colombo urban study.

Sample size was calculated using the Lwanga and Lameshow 1991 formula of n = z² p (100-p)D/ d². Expected prevalence of vitamin D deficiency and insufficiency/ dysglycaemia and obesity were taken as 50% and with design effect of 1.2%, precision of 95% and an anticipated 25% non-response, sample size was calculated as 600 using the EPI 6 sample calculation software.

A sample of 463 aged 18 years and above from the total population of 6473 in the GN area in three strata of the age categories was selected using stratified simple random sampling. The sample was divided among the 3 age categories of 18-40 years, 40-60 years and above 60 years on a weighted basis that took into account the proportion in the population and the expected prevalence of metabolic derangement / vitamin D deficiency, insufficiency in order to ensure the precision of the estimates in the sub sample analysis. Using a random
number generator, study subjects were randomly selected into the three age strata. The resulting disproportionate sample allocation was accounted for, by the use of weighted analysis. The weights were the inversion of the sampling fractions in the analysis.

**Data Collection:**

The participants were recruited at their homes by a team of researchers after providing an invitation letter and information documents. On the day of the screening, informed written consent was taken and data including socio-demographic data, use of alcohol, smoking, and detailed medical history on previous diagnoses and treatment were collected using interviewer-administered questionnaire by trained interviewers. Anthropometric measurements were made (weight, height, waist circumference, total body fat estimation and visceral fat percentage using a bio impedance analyzer-OMRON HBF 516). The following were measured in nine to twelve hours fasting stage: Plasma Glucose (GOD-PAP5 method, Olympus AU 480/680/400 analyser), Cholesterol (CHOD-PAP method, Olympus AU 480/680/400 analyser), Triglyceride (GPO-PAP method, Olympus AU 480/680/400 analyser), glycosylated hemoglobin (HPLC method, Bio-Rad Variant II Turbo analyser), Corrected Calcium (Arzenso III method, Olympus AU 480/680/400 analyser), and 25-OH Vitamin D level (Direct Chemiluminescence method, Advia centaur analyser). Vitamin D deficiency and insufficiency was defined according to IOM cut off values as less than 20ng/ml for deficiency and 20-30ng/ml for insufficiency (2).

**Statistical Analysis:**

Data analysis was performed in the R programming language version 3.2.2 (17). Community based prevalence with 95% confidence intervals for the urban study population and for different strata including age and gender were calculated considering the stratified sampling methodology using the “Survey” package in the R programming language. Descriptive data analysis was done and tabulated to present study population characteristics and prevalence of vitamin D deficiency/insufficiency.

Exploratory data analysis was done to identify the variables associated with vitamin D levels. The variables studied were age, gender, ethnicity, education level, smoking habits, alcohol consuming habits, diabetes and prediabetes, hyperlipidaemia, total cholesterol, low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL) and triglycerides (TG). Initially, each study variable was screened with simple linear regression, and the variables significant at P = 0.2 level were subsequently used for multiple variable analysis with multiple linear regression. Significant variables at multiple variable analysis were selected for the final model. The study variable Ethnicity had 4 categories (i.e. Sinhalese, Tamils, Moors and Other) where the “other” ethnicity had only 4 individuals and this group was not considered in reporting prevalence rates and analyzing interaction at the final model. P value of 0.05 were considered as significant.

**Ethical approval:**

Ethical approval was obtained from the Ethical Review committee of the Faculty of Medicine, University of Colombo. No invasive procedures used for this study and there was no anticipated risk to the participants. Documents were encoded to avoid any identifying character and measurements taken to ensure confidentiality of personal information.

**Results**

**Basic demographic and Metabolic characteristics of the study population:**

A total of 463 subjects completed the study and majority of participants were females (69%) across all age strata. There were 124 (70% females), 209 (73% females) and 130 (63% females) participants in the 18-40 years, 41-60 years and over 60-year age strata consecutively. The response rate in each of the above age strata was 59%, 87%, and 87% with an overall response rate of 77.2%. Current or ex-smokers accounted for 14.4% of total, while 20.1% were current or ex-alcohol consumers.

This population had a mean body Mass Index of 25.1 kg/m2 (SD 4.8), and 68.2% of the women and 59.1% of the men were overweight or obese based on South Asian criteria (18,19) Community prevalence for abdominal obesity was 58.1% based on International Diabetes Federation cut-off values on waist circumference for determining abdominal obesity in South Asians (WC – male >= 90cm, female >=80cm) (20). Mean total body fat percentage estimated with bio impedance analysis was 34.5 % (SD 10.5) and the estimated visceral fat was 9.5 % (SD 8.2). Estimated prevalence of Diabetes Mellitus was 27.6% (95% CI 23.7-31.4) and the age adjusted community prevalence was 27.1% based on the ADA/WHO criteria.
Prevalence of Vitamin D deficiency/Insufficiency:

Median (Interquartile range) 25(OH) D level of the study population was 18.9 (15.3 – 24.6) ng/ml (Figure 1). The estimated community prevalence of vitamin D deficiency was 58.8 (95% CI: 53.3-64.1) %, vitamin D insufficiency was 31.4 (26.4-36.5) % and cumulative prevalence of deficiency and insufficiency was 90.2 (87.2 – 93.2) %. Females had a higher vitamin D deficiency prevalence (65 (95% CI: 58.8 – 71.1) %) compared to males (45 (35.2 – 54.8) %). On the other hand, females’ vitamin D sufficiency prevalence (6.6 (3.6 – 9.6) %) was significantly less compared to males (16.8 (10.0 – 23.7) %). The vitamin D sufficiency in females over 60 years were only 3.5 (0.0 – 8.0) % which is significantly less than male over 60 years (30.3 (15.7 – 44.9) %). Interestingly, the highest vitamin D deficiency rate was noted in 18-40 age group at the community level (i.e. 65.9% (95% CI: 56.2 – 75.5)) and among males and females (Table 1).

Moors showed significantly less prevalence of vitamin D sufficiency compared to Sinhalese (3.4 (0.1 – 6.5) % vs 12.9 (8.7 – 17.1) %) Moors and Tamils had significantly lower vitamin D levels compared to Sinhalese but there was no difference between moors versus Tamils in vitamin D levels. People with family history of diabetes showed lower vitamin D levels and those who currently consumed alcohol and ex-alcohol consumers showed higher vitamin D levels compared to non-alcohol consumers but there was no significant difference between current alcohol consumers and ex-consumers.

Vitamin D deficiency/insufficiency and its demographic and Metabolic associations:

Population metabolic characteristics were analyzed according to their Vitamin D status. Body fat percentage was highest among vitamin D deficient group (37.4 (29.8 – 41.2) %) and lowest among vitamin D sufficient group (33.9 (28.0 – 38.0) %), but visceral fat percentage had not followed the same pattern. No major difference in BMI among these groups were noted. Unexpectedly, lowest waist circumference (84.0 (76.0 – 94.0)) was seen in Vitamin D deficient group and highest (89.2 (80.9 – 96.2)) was seen in Vitamin D sufficient group.

Mean lipid levels were highest (Total cholesterol - 208.8(182.6- 239.2), LDL - 121.5 (98.2 – 147.1), Triglycerides - 133.4 (95.6 – 171.8) in the Vitamin D deficient group, including HDL levels. (Table 2)

Study population was categorized into BMI categories according to South Asian and global cut offs and their Vitamin D levels were compared. Under both categorizations, obesity group had the lowest mean Vitamin D levels (19.73, 18.49), while underweight group had the highest mean Vitamin D levels (21.63, 21.63). Difference in Vitamin D levels among obese vs others was statistically significant when the global cut offs were used for categorization (Table 3)

Initial individual variable analysis with simple linear regression showed gender, ethnicity, family history of diabetes, smoking, alcohol consumption, waist circumference, body fat percentage and TG were significantly associated with vitamin D levels. Multiple linear regression analysis showed, ethnicity, family history of diabetes, alcohol consumption, waist circumference, and TG levels were significantly associated with vitamin D levels (Table 4).
### Table 1. Estimated community prevalence of vitamin D status

| Vit D deficiency | Vit D insufficiency | Vit D sufficiency |
|------------------|---------------------|-------------------|
| **Both sexes**   |                     |                   |
| Community        | 58.8 (53.5-64.1)    | 31.4 (26.4-36.5)  | 9.8(6.7-12.7)   |
| 18-40            | 65.9 (56.2-75.5)    | 28.4 (19.1-37.7)  | 5.7 (9.2-10.4)  |
| 41-60            | 52.1 (45.3-58.9)    | 35.3 (28.7-41.8)  | 12.6 (8.1-17.2) |
| 60+              | 58.9 (49.4-68.3)    | 27.8 (19.2-34.4)  | 13.3 (6.8-19.9) |
| **Males**        |                     |                   |
| Community        | 45.0 (35.2-54.8)    | 38.1 (28.6-47.8)  | 16.8 (10.0-23.7) |
| 18-40            | 48.1 (29.6-66.7)    | 40.7 (22.5-59.0)  | 11.1 (0.0-22.8) |
| 41-60            | 42.9 (30.4-55.3)    | 39.3 (27.0-51.6)  | 17.9 (8.2-27.5) |
| 60+              | 42.4 (26.7-58.1)    | 27.3 (13.1-41.4)  | 30.3 (15.7-44.9) |
| **Females**      |                     |                   |
| Community        | 65.0 (58.8-71.1)    | 28.4 (22.6-34.2)  | 6.6 (3.6-9.6)   |
| 18-40            | 73.8 (62.9-84.6)    | 23.0 (12.6-33.3)  | 3.2 (0.0-7.7)   |
| 41-60            | 56.0 (47.9-64.1)    | 33.6 (25.9-41.3)  | 10.4 (5.5-15.4) |
| 60+              | 68.4 (57.2-79.6)    | 28.1 (17.2-38.9)  | 3.5 (0.0-8.0)   |

### Table 2. Metabolic characteristics based on Vit D status

|                        | Vitamin D deficiency | Vit D insufficiency | Vit D sufficiency |
|------------------------|----------------------|---------------------|-------------------|
| **BMI**                | 26.2 (22.3 - 29.5)   | 25.0 (22.0 - 28.0)  | 26.6 (21.6 - 27.8) |
| **Waist circumference**| 84.0 (76.0 - 94.0)   | 87.0 (79.0 - 95.7)  | 89.2 (80.9 - 96.2) |
| **Neck circumference** | 33.0 (32.0 – 35.5)   | 34.0 (32.0 – 36.0)  | 34.0 (32.0 – 37.2) |
| **Total body fat**     | 37.4 (29.8 – 41.2)   | 35.2 (28.2 - 39.5)  | 33.9 (28.0 - 38.0) |
| **Visceral body fat**  | 9.0 (6.0 – 12.5)     | 9.0 (6.0 -12.0)     | 10.0 (4.0 – 10.0) |
| **TC**                 | 208.8(182.6- 239.2)  | 204.9(181.8- 238.7) | 205.5(163.8- 231.2) |
| **LDL**                | 121.5 (98.2 – 147.1) | 121.7 (93.4 – 151.9)| 113.1 (87.9 – 139.8) |
| **TG**                 | 133.4 (95.6 – 171.8) | 123.5 (94.1 – 155.4)| 107.5 (87.8 – 123.5) |
| **HDL**                | 59.2 (54.2 – 63.4)   | 59.8 (54.9 – 64.3)  | 57.7 (51.5 – 64.7) |
| **FBS**                | 88.4 (81.2 – 103.7)  | 90.3 (82.2 – 104.8) | 91.1 (84.1 – 99.3) |
| **HbA1c**              | 5.8 (5.5 – 6.6)      | 5.9 (5.5 – 6.8)     | 5.9 (5.5 – 6.4)    |
| **SBP**                | 120.0 (110.0 – 130.0)| 120.0 (110.0 – 130.0)| 120.0 (110.0 – 130.0)|
| **DBP**                | 80.0 (70.0 – 81.2)   | 80.0 (70.0 – 85.0)  | 80.0 (80.0 – 80.0) |
| **TSH**                | 1.5 (0.9 – 2.5)      | 1.3 (0.8 – 2.1)     | 1.2 (0.7 – 1.8)    |
| **Serum Calcium**      | 2.2 (2.1 – 2.3)      | 2.2 (2.1 – 2.3)     | 2.2 (2.1 – 2.3)    |
### Table 3. Vitamin D levels according to BMI categories

| South Asian BMI Categories | Mean Vit D level | Median Vit D level (Interquartile Range) | P value |
|----------------------------|------------------|------------------------------------------|---------|
| Underweight                | 21.63            | 18.71(14.89-23.70)                        |         |
| Normal                     | 21.23            | 19.77(16.27-25.47)                        |         |
| Overweight                 | 21.05            | 18.98(16.09-24.71)                        |         |
| Obese                      | 19.73            | 18.05(14.47-24.00)                        |         |
| Global BMI Categories      |                  |                                          |         |
| Underweight                | 21.63            | 18.71(14.89-23.70)                        |         |
| Normal                     | 20.95            | 19.28(16.07-24.90)                        |         |
| Overweight                 | 21.21            | 19.38(15.67-25.31)                        |         |
| Obese                      | 18.49            | 16.75(14.20-22.12)                        |         |

| Obese vs underweight       | 0.02045          |                                          |         |
| Obese vs normal            | 0.00999          |                                          |         |
| Obese vs overweight        | 0.05745          |                                          |         |

### Table 4. Parameter estimates

| Parameter                                      | Estimate     | Standard error | t value | P value |
|------------------------------------------------|--------------|----------------|---------|---------|
| Intercept                                      | 17.007392    | 2.272763       | 7.483   | <0.01   |
| Ethnicity                                      |              |                |         |         |
| Sinhalese vs Tamils                           | -1.708632    | 0.994072       | -0.176  | 0.09    |
| Sinhalese vs Moors                            | -3.250352    | 0.910397       | -3.570  | <0.01   |
| Sinhalese vs others                           | -0.804684    | 4.576572       | -0.176  | 0.86    |
| Family history of diabetes                    | -1.879279    | 0.690879       | -2.720  | <0.01   |
| Alcohol consumption                           |              |                |         |         |
| None vs current consumers                     | 4.146291     | 0.944381       | 4.390   | <0.01   |
| None vs ex-consumers                          | 5.369811     | 1.533552       | 3.502   | <0.01   |
| Waist circumference                           | 0.088292     | 0.026845       | 3.289   | <0.01   |
| Triglyceride levels                           | -0.023763    | 0.005116       | -4.644  | <0.01   |
Discussion

This study population with mixed ethnic and social characteristics from an urban setting in Sri Lanka showed very high cumulative estimated community prevalence of vitamin D deficiency and insufficiency (90.2% - 93.2%). This is comparable with South Asian regional prevalence (70-100%) (1-5) and clinical anticipation of high prevalence, while it is much higher than the reported prevalence of vitamin D deficiency in previously published data from Sri Lankan studies (8,9). Sun exposure is the major source of vitamin D, as very few naturally occurring food are rich in Vitamin D. This high prevalence despite ample sunshine throughout the year in the country could be possibly explained by the darker skin complexion, sunscreen use, less outdoor activities, lack of food fortification with vitamin D (6,7). Defining vitamin D deficiency based on international cut off may also play a role in finding high prevalence (21,22). Further studies on clinical significance of this biochemical diagnosis and defining local cut off and diagnostic criteria would be useful.

This problem was seen throughout all three age strata including younger population less than 40 years. Female gender and Moor ethnicity were recognized groups with high prevalence, but only Moor ethnicity was significantly associated with vitamin D deficiency. Clothing and other cultural, social factors associated with Moor ethnicity have been shown to be associated with high levels of vitamin D deficiency globally, especially in Muslim dominant countries in the South Asian region (1-5). Interestingly, it was found that vitamin D deficiency prevalence is highest among young, aged 18-40 years than older age group, where the efficiency of vitamin D biosynthesis is believed to decrease due to many factors. Some previous studies from Asia had showed similar results (23-25). This could be explained by rapid economic development and changing job patterns in recent past in urban setting which has resulted in young adults having indoor jobs, while elderly adults tend to have outdoor jobs as well have more time for other outdoor activities (24,25). This finding is significant as the peak bone mass which determines the future bone health is achieved at this age group.

In this population, alcohol consumption was positively associated with Vitamin D levels. Data concerning alcohol use and vitamin D remains controversial in literature. Recent literature review on this topic reported heterogeneous results, with a similar number of papers indicating a positive association, a negative association or the absence of any association between alcohol use and vitamin D levels (28). Several explanations have been given for negative association but exact biochemical explanation for positive association is not known. A speculative explanation given to this is that alcohol could suppress parathyroid hormone (PTH) levels and therefore 25 hydroxy Vitamin D (25OH vit D) is elevated as a result of reduced vitamin D activation in kidney, causing high measurable vitamin D levels (26).

Obesity is a known association of Vitamin D deficiency. In this population as well, obese category showed the lowest vitamin D levels compared to others. Their Vit D levels were significantly lower than in others, when global BMI cut offs, which are higher than the South Asian cut offs were used for categorization. Obesity and metabolic syndrome are emerging major health problems in the country at the moment (27,28) and this co existent Vitamin D deficiency will add to its health burden. Waist circumference, which is a measure of abdominal obesity did not correlate with Vit D levels in the expected manner in this population.

Total cholesterol, Triglyceride levels and LDL levels were highest in the Vit D deficient group, but only Triglyceride showed a significant association. This is a known association from Vitamin D studies conducted all around the world and this adverse association has been even seen among children with Vit D deficiency. Several mechanisms have been postulated for this. Serum Ca can reduce hepatic TAG production and secretion while low PTH levels could enhance peripheral TAG removal. Vitamin D deficiency associated low Ca absorption and PTH elevation are thought to cause high TAG levels (29,30). Other studies have also shown significant associations with total cholesterol, LDL and HDL with increased athrogenic risk (29).

Despite high prevalence of dysglycaemia, this study population did not show any significant association with the vitamin D status, which has been described in many observational studies and pre-clinical data over last decade (13-16). In that context it was hypothesized that vitamin D repletion would reduce Diabetes risk but results from randomized trials on this hypothesis were inconsistent (13-16). Recently published randomized, double-blind, placebo controlled D2d trial also showed no significant Diabetes risk reduction (31), and therefore it is not surprising to find no association between DM and vitamin D in our population.

Conclusion

This study reported very high prevalence of vitamin D deficiency and insufficiency in an urban setting in Sri Lanka across all age strata, which exceeds 90%. Moor ethnicity was the main recognized demographic association. Obese had significantly lower Vit D levels. Triglyceride levels were negatively correlated with vitamin D levels, but other metabolic derangements including diabetes showed no significant association with vitamin D status in the given population.

Clinical and skeletal outcomes of vitamin D deficiency/ insufficiency, as well, contribution of nutritional factors and extent of sun exposure for this condition should be extensively studied in the future with the view of planning interventions to
prevent this problem and replete the vitamin D status. Further studies to define local cut off and diagnostic criteria for vitamin D deficiency will be useful to identify the true burden of this problem on our population.

References

1. Van Schoor, N.M.; Lips, P. Worldwide Vitamin D Status. Best Pract. Res. Clin. Endocrinol. Metab. 2011, 25, 671–680.
2. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, et al. Evaluation, Treatment, and Prevention of Vitamin D Deficiency: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2011; 96(7):1911–1930.
3. Baidya, A.; Chowdhury, S.; Mukhopadhyay, S.; Ghosh, S. Profile of vitamin D in a cohort of physicians and diabetologists in Kolkata. Indian J. Endocrinol. Metab. 2012, 16, S416–S417.
4. Tandon, N.; Marwaha, R.K.; Kalra, S.; Gupta, N.; Dudha, A.; Koehupillai, N. Bone mineral parameters in healthy young Indian adults with optimal vitamin D availability. Natl. Med. J. India 2003, 16, 298–302.
5. Islam, M.Z., Shamim, A.A., Kemi, V., et al. (2008) Vitamin D deficiency and low bone status in adult female garment factory workers in Bangladesh. British Journal of Nutrition, 99, 1322-1329.
6. Clemens, T.L.; Adams, J.S.; Henderson, S.L.; Holick, M.F. Increased skin pigment reduces the capacity of skin to synthesise vitamin D3. Lancet 1982, 1, 74–76.
7. Matsuoka, L.Y.; Wortsman, J.; Haddad, J.G.; Kolm, P.; Hollis, B.W. Racial pigmentation and the cutaneous synthesis of vitamin D. Arch. Dermatol. 1991, 127, 536–538.
8. Meyer, H.E., Holvik, K., Loftius, C.M. and Tennakoon, S.U.B. (2008) Vitamin D status in Sri Lankans living in Sri Lanka and Norway. British Journal of Nutrition, 99, 941-944.
9. Rodrigo M, Hettiarachchi M, Liyanage C, Lekamwasam S. Low serum vitamin D among community-dwelling healthy women in Sri Lanka. Health. 2013;5(12): 1997-2003.
10. Parikh, S.J.; Edelman, M.; Uwaifo, G.I.; Freedman, R.J.; Semega-Janneh, M.; Reynolds, J.; Yanovski, J.A. The relationship between obesity and serum 1,25-dihydroxy vitamin D concentrations in healthy adults. J. Clin. Endocrinol. Metab. 2004, 89, 1196–1199.
11. Lagunova, Z.; Porojnicu, A.; Lindberg, F.; Hexeberg, S.; Moan, J. The dependency of vitamin D status on body mass index, gender, age and season. Anticancer Res. 2009, 29, 3713–3720.
12. Jacobo Wortsman, Lois Y Matsuoka, Tai C Chen, Zhiren Lu, and Michael F Holick. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr. 2000;72:690–3.
13. Pittas, A.G.; Lau, J.; Hu, F.B.; Dawson-Hughes, B. The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. J. Clin. Endocrinol. Metab. 2007, 92, 2017–2029.
14. Afzal S, Bojesen SE. Low 25-Hydroxyvitamin D and Risk of Type 2 Diabetes: A Prospective Cohort Study and Metaanalysis. Clinical Chemistry.2013; 59:2 381–391.
15. R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
16. World Health Organization. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet. 2004; 363:157–63
17. Somasundaram N, Rajaratnam H, Wijeyaratne C et al. Clinical guidelines: The Endocrine Society of Sri Lanka: Management of Obesity. Sri Lanka Journal of Diabetes, Endocrinology and Metabolism 2014; 1: 55-70.
20. Alberti KGMM, Zimmet PZ, Shaw JE. The metabolic syndrome—a new world-wide definition from the International Diabetes Federation consensus. Lancet. 2005; 366:1059-62.

21. Manson JE, Brannon PM, Rosen CJ, Taylor CL. Vitamin D Deficiency - Is There Really a Pandemic? N Engl J Med. 2016 Nov 10;375(19):1817-1820.

22. Selvarajan S, Gunaseelan V, Anandabaskar N, et al. Systematic Review on Vitamin D Level in Apparently Healthy Indian Population and Analysis of Its Associated Factors. Indian J Endocrinol Metab. 2017;21(5):765–775.

23. Surekha Bhat M, Lasrado I, Rajeshwari SG, Gururaja A, Prabhu K, et al. (2017) Prevailing Serum Vitamin D Levels among Individuals in South Karnataka. J Bioanal Biomed 9:184-188. doi: 10.4172/1948-593X.1000178

24. Choi HS, Oh HJ, Choi H, Choi WH, Kim JG, Kim KM, et al. Vitamin D insufficiency in Korea—a greater threat to younger generation: the Korea National Health and Nutrition Examination Survey (KNHANES) 2008. J Clin Endocrinol Metab. 2011;96:643–51. doi: 10.1210/jc.2010-2133.

25. Chailurkit LO, Aekplakorn W, Ongphiphadhanakul B. Regional variation and determinants of vitamin D status in sunshine-abundant Thailand. BMC Public Health. 2011;11:853. doi: 10.1186/1471-2458-11-853.

26. Tardelli, V. S., Lago, M. P. P. do, Silveira, D. X. da, & Fidalgo, T. M. (2017). Vitamin D and alcohol: A review of the current literature. Psychiatry Research, 248, 83–86.

27. Katulanda P, Jayawardena MA, Sheriff MH, Constantine GR, Matthews DR. Prevalence of overweight and obesity in Sri Lankan adults. Obes Rev. 2010;11:751-756

28. Katulanda P, Ranasinghe P, Jayawardena R, Sheriff R, Matthews DR. Metabolic syndrome among Sri Lankan adults: prevalence, patterns and correlates. Diabetol Metab Syndr. 2012;4:24

29. Chaudhuri JR, Mridula KR, Anamika A, et al. Deficiency of 25-hydroxyvitamin d and dyslipidemia in Indian subjects. J Lipids. 2013;2013:623420.

30. Rodríguez-Rodríguez E, Ortega RM, González-Rodríguez LG, López-Sobaler AM. Vitamin D deficiency is an independent predictor of elevated triglycerides in Spanish school children. Eur J Nutr. 2011 Aug;50(5):373-8.

31. Pittas AG, Dawson-Hughes B, Shechen P, et al. Vitamin D supplementation and prevention of type 2 diabetes. N Engl J Med. DOI: 10.1056/NEJMoal900906.

Declarations:

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Ethics approval and consent to participate

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Competing interests

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Authors’ contributions

NPS, KG involved in designing the study and data collection. CJS and DE analysed the data. CJS drafted the manuscript and all other authors read and approved it.

Availability of data and materials

The data analysed in this paper can be made available to researchers. Requests for access to the data set used in this paper should be directed to the corresponding author.
Serum c-peptide level in newly diagnosed Bangladeshi adults with type 2 diabetes mellitus

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Abstract

C-peptide is produced in equimolar amounts to insulin and is the best measure of endogenous insulin secretion. This cross-sectional study was conducted in a tertiary hospital of Bangladesh from January 2016 to December 2017 to assess fasting serum c-peptide as a marker of the endogenous insulin secretory capacity in newly diagnosed subjects with type 2 diabetes mellitus (T2DM). 60 newly diagnosed T2DM subjects were investigated along with 30 age-sex matched healthy controls. Fasting c-peptide was significantly higher in T2DM subjects than controls (8.97±5.96 vs. 1.69±0.66 ng/ml). None of the T2DM subjects had subnormal c-peptide, 19 (32%) had normal c-peptide, and 41 (68%) of them had elevated c-peptide levels. Higher fasting plasma glucose (FPG), plasma glucose 2-hours after 75gm oral glucose tolerance test (PG 2H-OGTT) and HbA1c levels were observed in T2DM subjects with elevated c-peptide in comparison to T2DM subjects having normal c-peptide. In T2DM subjects, c-peptide showed significant positive correlations with body mass index (BMI), FPG, PG 2H-OGTT, and HbA1c. This study found higher levels of fasting c-peptide in newly diagnosed T2DM in comparison to nondiabetic controls. None of the T2DM subjects had a subnormal c-peptide level.

Keywords: type 2 diabetes, endogenous insulin, c-peptide, insulin secretion

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Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. More than 90% of subjects with DM worldwide have type 2 diabetes (T2DM) (1). Though insulin resistance in the liver & muscle and β-cell failure represent the core pathophysiological defects in T2DM, it now is recognized that the β-cell failure occurs much earlier and is more severe than previously thought (2). In Asia, the T2DM phenotype appears to be somewhat different from that in the western countries with a younger age of onset and with lower BMI while associated with greater visceral adiposity and reduced insulin secretory capacity (3).

Many studies have evaluated serum c-peptide as a marker of insulin secretion in T2DM subjects (4,5,6,7,8). Data regarding the β-cell secretory state in newly detected Bangladeshi T2DM patients are scarce. The current study was undertaken to address the deficit.

Methods

This cross-sectional study was conducted in the Department of Medicine of M.A.G. Osmani Medical College Hospital, Sylhet, Bangladesh from January 2016 to December 2017, with the permission of the institutional review board of the hospital. All newly diagnosed patients with T2DM attending the Medicine Outpatient Department of the hospital during the study period were considered as the study population. 60 newly diagnosed non-pregnant adult patients with T2DM, age 35 to 65 years, before initiation of any lifestyle modification or pharmacological treatment for DM, were selected by non-probability convenient sampling technique. 30 age and sex-matched otherwise healthy volunteers selected from the patients’ attendants and health care professionals were included in the control group. Subjects with any acute illness, any acute or advanced chronic complications of DM were excluded. Informed written consent was taken from each study subject before enrollment; relevant history was taken, physical examinations including anthropometric measurements were done; collected data were recorded in a pre-specified data collection sheet. Obesity status was determined by body mass index (BMI) categories applicable to the Asian Indians and waist circumference ≥90 cm in male and ≥80 cm in female were used to define abdominal obesity (9,10). All of the participants were asked to attend the OPD on another convenient day with overnight fasting for at least 8 hours and all attending patients underwent standard oral glucose tolerance test (OGTT) according to the procedure described by World Health Organization (11). Diabetes mellitus was diagnosed according to the American Diabetes Association (ADA) criteria (1). The fasting blood sample was also used for estimation of HbA1c, serum c-peptide, serum creatinine, and lipid profile.

Biochemical Analysis:

Plasma glucose, serum creatinine, and fasting lipid profile was estimated by a semi-auto analyzer (Screen Master 3000 manufacturer: Biochemical System International, Italy), HbA1c was assayed by immunofluorescence assay on NGSP certified quantitative immunoassay analyzer Getein 1100 (Getein Biotech, Inc, China), serum c-peptide was assayed by quantitative ELISA method (DRG C-Peptide) with ELISA reader plate (DAS srl, Italy). All the biochemical analyses were done in the Department of Biochemistry of the hospital.

Statistical analysis:

Statistical analysis was done using Statistical Packages for Social Sciences (SPSS) software version 23.0 (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0, Armonk, NY: IBM Corp.). The categorical variables were represented as percentages and measurable variables as mean±standard deviation (SD). Student’s t-test and Chi-square test were performed as applicable for comparing the variables between different groups. Pearson’s correlation test was used to observe the correlation of c-peptide level with other variables. p-value ≤0.05 was considered to be statistically significant.

Results

Demographic, clinical and biochemical characteristics of the study population are shown in table 1. The T2DM group and control group subjects did not differ in respect of age, sex, and serum creatinine levels. T2DM subjects had a higher frequency of first-degree relatives with T2DM; BMI and waist-to-hip ratio was higher in the T2DM group than the control group. The c-peptide level was also higher in T2DM subjects than controls.
Table 1. Demographic, clinical and biochemical characteristics of the study participants

| Variables                        | T2DM (n=60)                      | Control (n=30)                    | p     |
|----------------------------------|----------------------------------|----------------------------------|-------|
|                                  | Mean ± SD or %                   | Mean ± SD or %                   |       |
| Age (years)                      | 45.0 ± 6.5                       | 42.6 ± 3.9                       | 0.070a|
| Gender                           |                                  |                                  |       |
| Male                             | 75%                              | 80%                              | 0.597b|
| Female                           | 25%                              | 20%                              |       |
| Have family H/O T2DM             | 65%                              | 40%                              | 0.024b|
| BMI (Kg/M2)                      | 25.79 ± 2.27                     | 24.64 ± 1.40                     | 0.012a|
| Waist:Hip Ratio                  | 0.95 ± 0.02                      | 0.94 ± 0.01                      | 0.034a|
| Fasting Plasma Glucose (mg/dL)   | 155.53 ± 65.69                   | 94.13 ± 9.05                     | <0.001a|
| Pl. Glucose 2 hour after OGTT (mg/dL) | 277.98 ± 91.29                  | 117.47 ± 17.26                   | <0.001a|
| HbA1c (%)                        | 8.72 ± 2.17                      | 5.65 ± 0.35                      | <0.001a|
| S. Creatinine (mg/dL)            | 1.02 ± 0.17                      | 1.09 ± 0.16                      | 0.071a|
| Total Cholesterol (mg/dL)        | 188.19 ± 30.99                   | Not done                         |       |
| Triglyceride (mg/dL)             | 190.90 ± 68.68                   | Not done                         |       |
| LDL-Cholesterol (mg/dL)          | 112.03 ± 22.34                   | Not done                         |       |
| HDL-Cholesterol (mg/dL)          | 37.42 ± 2.88                     | Not done                         |       |
| C-peptide (ng/mL)                | 8.97 ± 5.96                      | 1.69 ± 0.66                      | <0.001a|

BMI = Body mass index; *by Student’s t-test; †by Chi-square Test

Table 2. Comparison of BMI, fasting plasma glucose, plasma glucose 2hrs after OGTT, and HbA1c between T2DM subjects with normal and high c-peptide

| Variables                        | Normal C-Peptide (0.5-3.2 ng/ml) (n=19) | High C-Peptide (>3.2 ng/ml) (n=41) | p     |
|----------------------------------|------------------------------------------|-----------------------------------|-------|
|                                  | Mean ± SD                                | Mean ± SD                         |       |
| BMI (Kg/M2)                      | 25.77 ± 2.14                             | 25.90 ± 2.33                      | 0.838 |
| Fasting Plasma Glucose (mg/dL)   | 124.21 ± 35.34                           | 170.05 ± 71.55                    | 0.011 |
| Pl. Glucose 2 hour after OGTT (mg/dL) | 234.95 ± 74.65                           | 297.93 ± 92.17                    | 0.012 |
| HbA1c (%)                        | 7.53 ± 1.90                              | 9.26 ± 2.07                       | 0.003 |

BMI = Body mass index; p-value by Student’s t-test
Table 3. Correlations of c-peptide with other variables in T2DM subjects

| Variables                                   | r value | p value |
|---------------------------------------------|---------|---------|
| Age (years)                                 | 0.068   | 0.607   |
| BMI (Kg/M2)                                 | 0.337   | 0.008   |
| Waist-to-Hip ratio                          | 0.179   | 0.170   |
| Serum creatinine (mg/dL)                    | -0.013  | 0.919   |
| Fasting Plasma Glucose (mg/dl)              | 0.436   | 0.001   |
| Pl. Glucose 2 hour after OGTT (mg/dL)       | 0.402   | 0.001   |
| HbA1c (%)                                   | 0.367   | 0.004   |

by Pearson correlation

Table 2 shows the metabolic parameters of the T2DM subjects with normal and high c-peptide levels.

Correlations of serum c-peptide level with other variables in T2DM subjects are given in table 3. Serum c-peptide showed significant positive correlations with BMI, fasting plasma glucose, plasma glucose 2 hours after OGTT, and HbA1c.

Discussion

T2DM is one of the leading causes of morbidity and mortality globally. While all ethnic groups are affected, the prevalence of T2DM in South Asians is extremely high and is continuing to rise rapidly. Though the South Asians share the basic pathophysiological defects of T2DM observed in other ethnic groups, there is strong evidence to suggest that South Asians are more insulin resistant than Caucasians with the onset of diabetes at younger ages and with comparatively lower BMI. In addition to an increased propensity for insulin resistance, South Asians may also experience early declines in β-cell function compared with other ethnic groups and an early impairment in β-cell function could also be a key pathophysiological mechanism in T2DM development in South Asians.(12)

There are different methods to measure β-cell secretory function. Acute insulin response (AIR) or AIRmax is the gold standard for assessment of β-cell function but difficult to perform in a clinical setting (13). Assay of serum insulin as a measure of insulin secretion has several limitations as insulin has a half-life of 3-5 minutes and almost half of all insulin secreted by the pancreas is degraded by hepatic first-pass metabolism. So, peripheral insulin concentration reflects post-hepatic insulin delivery rather than the actual secretory rates of insulin. C-peptide secreted in the equimolar amount of insulin has negligible extraction by the liver and constant peripheral clearance making its half-life longer than insulin. For these reasons, it is commonly used in preference to insulin measurement when assessing β-cell function in clinical practice (14).

The current study assessed the endogenous insulin secretory capacity of the participants by measuring fasting serum c-peptide. None of the newly diagnosed T2DM subjects had a c-peptide level below the normal range. C-peptide was higher in new T2DM subjects in comparison to the non-diabetic otherwise healthy controls (8.97 ± 5.96 vs. 1.69 ± 0.66 ng/ml, p<0.001). This indicates that the study subjects had no absolute reductions in insulin secretion; there was a compensatory increase in insulin secretion in many to overcome the insulin resistance which was not measured in this study.

In this study, 41 (68%) of the T2DM subjects had elevated (>3.2ng/ml) fasting c-peptide level and the rest 19 (32%) had normal level (0.5-3.2 ng/ml) of c-peptide. Higher level c-peptide indicates the potential good response to insulin sensitizers and other oral anti-diabetic drugs (14). The mean HbA1c of the studied T2DM subjects was 8.72% (±2.17). Kamrul-Hasan et al. found higher HbA1c (10.69% ± 2.64) in newly diagnosed Bangladeshi T2DM subjects in another study (15). The HbA1c level of this study subjects indicates that their diabetes can be controlled by metformin plus another second line oral anti-diabetes drug without insulin use (14). The high c-peptide group of the T2DM subjects had significantly higher levels of FPG, plasma glucose 2 hours after OGTT, and HbA1c than the normal c-peptide group.
group. In the initial stage of T2DM, when blood glucose rises there is a compensatory rise of insulin secretion in a proportionate manner to keep the blood glucose within the normal range. If exaggerated insulin secretion cannot overcome the insulin resistance, hyperglycemia occurs (2). Higher glycemic indices in high c-peptide T2DM subjects match the pathophysiology of T2DM. The c-peptide level was found to have significant positive correlations with FPG, plasma glucose 2 hours after OGTT, and HbA1c in T2DM subjects. Higher mean FPG and HbA1c in patients with a higher c-peptide level and positive correlations of c-peptide with FPG and HbA1c were also observed by Deep et al.(5). Abdullah et al. and Mariyam et al. also observed positive correlations between c-peptide level and FPG (6,7).

Though no difference was observed in BMI between T2DM subjects with high c-peptide level and normal c-peptide level, a significant positive association was found between c-peptide and BMI in the studied T2DM subjects. Obesity is associated with insulin resistance and more insulin secretion is needed to overcome the higher level of insulin resistance in obese subjects. Mariyam et al. had similar observations (7).

Conclusion
Insulin secretion estimated by measurement of fasting c-peptide was either normal or high in newly diagnosed T2DM subjects in the current study indicating a predominant role of insulin resistance in the etiology of T2DM in Bangladeshi subjects. Further research can explore the exact contribution of insulin resistance and insulin secretory defects in this area.

Limitations of the study:
Our sample size was small and randomization of sampling was not done. This was a single tertiary level hospital-centered study so the result may not reflect the whole community. Insulin resistance was not measured so the relative insulin deficiency could not be demonstrated.

Conflicts of interest:
None.
References

(1) American Diabetes Association. 2. Classification and diagnosis of diabetes: Standards of Medical Care in Diabetes. 2019. Diabetes Care. 2019;42(Suppl. 1):S13–S28

(2) DeFronzo RA. From the Triumvirate to the Ominous Octet: A New Paradigm for the Treatment of Type 2 Diabetes Mellitus. Diabetes. 2009;58(4):773-95.

(3) Saisho Y. β-cell dysfunction: Its critical role in prevention and management of type 2 diabetes. World Journal of Diabetes. 2015;6(1):109-24.

(4) Neha, Sharma A, Kaur J, Uppal V, Singh I. Correlation of Serum C-Peptide and Serum uric acid Levels with Glycated Hemoglobin in Patients of Type 2 Diabetes Mellitus. International Journal of Clinical Biochemistry and Research. 2016;3(3):330-4.

(5) Deep HS, Singh BP, Singh SP. Evaluation of serum C-Peptide levels in type 2 diabetes in Punjabi population. International Journal of Advances in Medicine. 2017;4(4):1026-30.

(6) Abdullah BB, Patil BS, Thaseen A. Significance of C Peptide on T2DM-A Study in the North Kornataka population in India. Al Ameen J Med Sci. 2010;3(1):65-78.

(7) Mariyam SB, Muthubevi SB, Vasantha SC. Serum C-Peptide level in obese and non-obese patients with type 2 diabetes mellitus. J Evolution Med Dent Sci. 2017;6(5):350-3.

(8) Tajiri Y, Kimura M, Mimura K, Umeda F. Variation of fasting serum C-Peptide level after admission in Japanese patients with type 2 diabetes mellitus. Diabetes Technology & Therapeutics. 2009;11(9):593-9.

(9) WHO expert consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. The Lancet. 2004;363(9403):157-63.

(10) The IDF consensus worldwide definition of metabolic syndrome. Guideline for definition of the metabolic syndrome. International Diabetes Federation. 2006.

(11) World Health Organization: Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: Report of a WHO/IDF consultation. Geneva, Switzerland: World Health Organization. 2006.

(12) Gujral UP, Pradeepa R, Weber MB, Narayan KM, Mohan V. Type 2 diabetes in South Asians: similarities and differences with white Caucasian and other populations. Ann N Y Acad Sci. 2013;1281(1):51-63.

(13) Cobelli C, Toffolo GM, Man CD, Campioni M, Denti P, Caumo A, et al. Assessment of β-cell function in humans, simultaneously with insulin sensitivity and hepatic extraction, from intravenous and oral glucose tests. Am J Physiol Endocrinol Metab. 2007;293(1):E1-15.

(14) Leighton E, Sainsbury CAR, Jones GC. A Practical Review of C-Peptide Testing in Diabetes. Diabetes Ther. 2017;8(3):475-87.

(15) Kamrul-Hasan ABM, Fariduddin M, Ghosh DK, Moinul-Islam, Atikur-Rahman M, Nusrat-Sultana, et al. Vitamin B12 is Found Sufficient in Newly Diagnosed Type 2 Diabetes in a Hospital Based Study. Int J Diabetes Metab Disord. 2016;1(1):1-5.
Serum non-high-density lipoprotein cholesterol concentration: Profile and prevalence of high levels among adolescent Nigerian students.

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Abstract

Background/Aim: Non-high-density lipoprotein cholesterol (non-HDL-C) concentration include both cholesterol-rich and triglycerides-rich atherogenic apolipoprotein B-containing lipoproteins. The present study described the distribution and estimated the prevalence of high serum non-HDL-C levels in adolescent Nigerian students.

Methods: This was a case control study, conducted in an urban secondary school. The study population consists of a total of 98 students, aged 10-16 years (49 subjects with high BMI (≥ 85th percentile) and 49 controls with normal BMI (5th to < 85th percentile). The first school in an alphabetically arranged list was selected. The participants were randomly selected and matched for age and sex. The serum concentrations of total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), triglycerides (TG) and low-density lipoprotein-cholesterol (LDL-C) were determined, using automated analyzer with commercially available kits. The serum non-HDL-C concentration was determined from the difference between the TC and HDL-C concentrations.

Results: The prevalence of elevated (borderline plus abnormal) non-HDL-C concentrations in overweight/obese and normal-weight participants were 26.5% (95% CI=26.3-26.6) and 8.2% (95% CI = 5.9-14.5) respectively; Z-test statistic = 2.666, p < 0.01. In overweight/obese participants, the prevalence rates of “borderline” and “abnormal” serum non-HDL-C levels were 24.5% and 2.0%, respectively. The corresponding prevalence rates in normal-weight participants were 8.2% and zero percent, respectively. The frequency of high non-HDL-C was higher in participants whose waist-to-height ratio (WHtR) was ≥ 0.5 than those with WHtR < 0.5. The risk of high serum non-HDL-C level was 2.5-fold higher in girls than boys. The mean serum non-HDL-C concentrations in overweight/obese and normal weight participants were 101.4±23.3mg/dl and 93.6±24.8mg/dl, respectively; p>0.05. The mean serum non-HDL-C in overweight/obese boys was 3.9mg/dl higher than that of normal weight boys and for the girls, this difference was 10.8mg/dl.

Conclusion: The risk of developing a significantly elevated non-HDL-C level is greater in overweight/obese than normal-weight adolescents and this risk is also greater in girls.

Keywords: Adolescence, non-high-density lipoprotein cholesterol, obesity, overweight, Nigeria.

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Introduction

The non-high-density lipoprotein cholesterol (non-HDL-C) is estimated as total serum cholesterol minus the high-density lipoprotein cholesterol concentration. It reflects the total mass of cholesterol transported in atherogenic apo B-containing lipoproteins with approximately three-quarter of the cholesterol as low-density lipoprotein (LDL) and one-quarter as very-low-density lipoprotein cholesterol (VLDL) (1). Several recent studies have confirmed the importance of non-HDL-C as a reliable, easy to calculate parameter that is strongly correlated with cardiovascular disease risk in adulthood (2-4) but limited studies have been conducted in the paediatric age group. In addition, the determination of non-HDL-C does not attract an extra cost. The serum levels of non-HDL-C have been found to be related to body mass index and waist circumference (4). In one study, it was found that the difference between median values of serum non-HDL-C levels of severely obese children and those of their normal weight counterparts were approximately 20 to 30mg/dl (5).

Corey et al (6) proposed that non-HDL-C is a useful biomarker for nonalcoholic steatohepatitis. A recent study involving US children and adolescents aged 12-19 years found that elevated non-HDL-C level is associated with metabolic syndrome [7]. Recently, non-HDL-C has been established to be superior to LDL-C as a predictor of CVD risk [3,4]. Sniderman et al [8], in a meta-analysis involving 233,455 subjects and 22,950 events confirmed the superiority of non-HDL-C over LDL-C in the prediction of CVD risk. Assessment of serum levels of non-HDL-C has been recommended as a target for therapy and this recommendation was based on the observation that post-prandial chylomicronemia minimally influences the relationship between non-HDL-C levels and coronary heart disease risk (9). In recognition of the usefulness of non-HDL-C as a biomarker of CVD risk, the American Academy of Pediatrics (AAP) in 2011, redefined dyslipidaemia by adopting the non-HDL-C level (instead of the LDL-C level), triglyceride level and HDL-C level as three major criteria for its definition [10]. In an editorial, Abe (11) concluded by recommending the use of serum levels of non-HDL-C for childhood cholesterol screening.

Only few studies have examined the prevalence of elevated serum non-HDL-C levels in the paediatric age group. In this regard, the results of a study in USA involving children and adolescents showed that the prevalence of high serum non-HDL-C level varied from 11.8% to 15.0%, depending on age, gender, ethnicity and weight status (12). Among Chinese children and adolescents, the reported prevalence of high serum non-HDL-C was 4.1% (13). In literature, there are no reports from African countries (Nigeria inclusive) regarding prevalence of high serum non-HDL-C in children and adolescents, despite its usefulness as a biomarker of CVD risk in adulthood. It has been established that CVD risk factors occur in clusters and tend to track from childhood to adulthood (14). Therefore, preventive measures should begin from childhood in order to delay progression to clinical disease. The purpose of the present study was to describe the distribution and estimate the prevalence of high serum non-HDL-C levels in a selected group of overweight/obese and normal-weight adolescent Nigerian students.

Participants and methods

This was a cross-sectional study carried out in an urban secondary school in Egor Local Government Area (LGA) of Edo State, Nigeria. The study was conducted from 1st to 30th June, 2016. Ethical clearance certificate was obtained from Research and Ethics Committee of the College of Medical Sciences, University of Benin, Benin City. A written consent was obtained from the parent(s)/guardian of each of the selected students. Each study participant gave a verbal consent for the study. We emphasized to the students that their participation was entirely voluntary. In addition, permission was obtained from the administrative head of the school and the ministry of education.

Study group and sampling technique

In this study, the first private secondary school in a list alphabetically arranged secondary schools in Egor, Local Government Area (LGA) was selected. Thereafter, the students were randomly selected. Each of the selected students was given a written note explaining the nature of the study as well as a questionnaire to be completed by their parent(s). The subjects (high BMI, ≥ 85th percentile) and controls (normal BMI, 5th to < 85th percentile) were matched for age and gender. The socioeconomic status and ethnicity of the subjects and controls were similar in all respects as the school was a public school where there is no discrimination. The students attending the school were from various Nigerian social strata and ethnicity. Based on clinical assessment, any student with a positive history or obvious clinical evidence of hypothyroidism, liver disease, chronic kidney disease, Cushing syndrome, diabetes mellitus or who is on drugs such as corticosteroids or oral contraceptives were excluded from the study.

Anthropometric measurements

Using the procedure recommended by Marfell-Jones et al (15), the height was measured to the nearest 0.1cm, using a Holtian portable anthropometer and the weight was measured to the nearest 0.1kg, using a Seca Scale Balance with the subject in light clothing and bare foot. The waist circumference was measured at the midpoint...
between the upper border of the iliac crest and lowest border of the last rib, at the end of normal expiration. If a duplicate measurement differed by > 0.5cm or > 0.5kg respectively, a third measurement was performed and the average of the two closest measurements was recorded as the final value. To eliminate inter-observer error, all the anthropometric measurements were performed by one of the authors in the presence of a chaperone. The body mass index of each of the subjects was computed, using the standard formula (15).

Blood sample collection and serum lipid profile analysis

The details of the intended procedure was explained to each of the participants before collection of blood sample. Prior to sampling, the participants maintained their usual dietary pattern within the past 3 days preceding the study. Using an aseptic technique, a venous puncture in the antecubital fossa was performed and 10ml of blood was collected into appropriate sample container without anticoagulant and stored at 8OC. After one hour, all samples were centrifuged at 3000rpm for 15 minutes and the serum aliquots were stored at -70OC until assayed. The serum concentrations of total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C) and triglycerides (TG) and low-density lipoprotein-cholesterol (LDL-C) were determined, using automated analyzer with commercially available kits and following strictly the manufacturer's instructions throughout the assay procedures.

Definitions

The non-HDL-C concentration was calculated as the TC concentration minus the HDL-C concentration (10). In the present study, we used the National Heart, Lung and Blood Institute (NHLBI) Expert Panel on Integrated Guidelines for Cardiovascular Health Risk Reduction in Children and Adolescents criteria to define high serum non-HDL-C concentration. NHLBI categorized high serum non-HDL-C into (i) borderline(120-144mg/dl) and abnormal (≥145mg/dl) (10). Normal weight, overweight and obesity were defined by BMI between 5th to < 85th, 85th to < 95th and ≥ 95th percentiles, respectively. Central (abdominal) obesity was defined by waist-to-height ratio (WHtR) ≥ 0.5 (16). The three phases of adolescence are early adolescence (10-13 years old); middle adolescence (14-16 years old); and late adolescence 17 years old and above) (17).

Statistical analysis

The data were collated and entered into an Excel spread sheet. Accuracy of the data entered was double checked. Subsequently, data were analysed, using Microsoft Excel and SPSS (Statistical Package for Social Sciences) version 20.0. Measures of central tendency and dispersion involving the mean and standard deviation were computed for all quantitative data. Confidence intervals, odds ratio, frequency distribution and percentages were calculated. Differences between means and prevalence were tested, using t- and Z-tests, respectively. Significant p-value was set at < 0.05.

Results

A total of 98(49 overweight/obese, subjects and 49 normal weight, controls) were studied, comprising 21 boys and 28 girls in each of the two groups. The mean age of the participants was 12.9±1.2 years, ranging between 10 and 16 years. The clinical characteristics of the participants are presented in Table 1. The mean BMI, waist-to-height and waist-to-hip ratios were separately significantly higher in overweight/obese than normal weight group. The serum non-HDL-C levels ranged from 61.7mg/dl to 156.6mg/dl in overweight/obese group and 63.9mg/dl to 135.9mg/dl in normal-weight group. As shown in Table 2, the prevalence of elevated (borderline plus abnormal) non-HDL-C concentrations was significantly higher in overweight/obese than normal-weight participants, 26.5% (95% CI=26.3-26.6) and 8.2% (95% CI = 5.9-14.5) respectively; Z-statistic = 2.566, p < 0.01. Similarly, the prevalence of borderline-high non-HDL-C concentrations was significantly higher in overweight/obese than normal-weight participants, 24.5% (95% CI= 24.4-24.6) and 8.2% (95% CI = 5.9-14.5) respectively; Z-statistic = 3.037, p < 0.001. The only participant with a very high serum non-HDL-C level (156.6mg/dl) was an 11-year-old girl with waist circumference 96.5cm (> 97th percentile), hip circumference of 101.6cm (> 97th percentile), giving a waist/hip circumference ratio of 0.95. Her blood pressure was 110/80mmHg (systolic < 90th percentile and diastolic 95th percentile) and BMI 26kg/m2 (> 95th percentile). Table 3 shows prevalence of elevated non-HDL-C was significantly higher in participants whose waist-height ratio (WHtR) was ≥ 0.5 than those whose WHtR was < 0.5. The mean serum non-HDL-C concentrations in overweight/obese and normal weight participants were 101.4±23.3mg/dl and 93.6±24.8mg/dl, respectively; t-statistic 1.114, p>0.05. Table 4 shows that the mean serum non-HDL-C in overweight/obese boys was 3.9mg/dl higher than in their normal weight counterparts. In addition, comparing overweight/obese boys and girls, the difference in mean serum non-HDL-C was 11.1mg/dl higher in girls. The risk of high serum non-HDL-C level was 2.5-fold greater in girls than boys. In both overweight/obese and normal weight participants, the mean serum non-HDL-C concentrations was higher in females than males.
Discussion

In consonance with the reports of previous studies [4,9,10], data from the present study indicate that the prevalence of elevated (borderline plus abnormal) non-high-density lipoprotein cholesterol was significantly higher in overweight/obese than normal-weight adolescents. This finding suggests that overweight/obesity is a risk factor for development of dyslipidaemia in adolescence. Given that dyslipidaemia is a component of metabolic syndrome, it follows that overweight/obese adolescents are at higher risk of metabolic syndrome than their normal weight counterparts. This view is supported by the results of the study by Li et al. (7) among US youths aged 12-19 years that demonstrated increased risk of metabolic syndrome in overweight/obese subjects. The prevalence (26.5%) of high (borderline plus abnormal) non-HDL-C levels found in the present study was comparable to 26.0% reported among Iranian students aged 11 to 18 years (18) but slightly lower than 32.5% found among Mexican adolescents (19). One-quarter of overweight/obese participants in this study had borderline-high non-HDL-C, which was in general agreement with 18.9% reported from Iran (18). Further comparison was not possible because of scarcity of published studies that have examined the subject in paediatric population. Considering the strong association between elevated serum non-high-density lipoprotein level and development of metabolic syndrome, it is reasonable to advocate for initiation of health education related to consumption of healthy diet and promotion of exercise, particularly among adolescents students with borderline-high serum non-HDL-C value. This view is supported by the fact that cardiovascular disease risk factors are known to occur in clusters and tend to track from childhood to adulthood (14). Two percent of our subjects were in the “abnormal” category of high serum non-HDL-C level and this was lower than 4.1% and 8.1% reported among Chinese and Iranian adolescents, respectively (13,18).

With regard to gender, the risk of high serum non-HDL-C level was 2.5-fold higher in girls than boys. This is consistent with the findings of previous studies (4,13). Although Al-Dagharti et al (20) reported a higher risk of high non-HDL-C in girls, they also noted that high serum non-HDL-C concentrations was associated with more cardiometabolic risks in boys than girls. The higher frequency of high non-HDL-C in girls may be explained by the observed higher prevalence of overweight/obesity in girls than boys, both in the present study as well as in others (4,12). This is expected, given that overweight/obesity is a known risk factor for high serum non-HDL-C levels in the paediatric population (5,12).

Data from the present study showed that amount of body fat as measured by BMI and waist-to-height ratio (WHtR) was positively associated with serum non-HDL-C levels. The mean serum non-HDL-C was significantly higher in overweight/obese group than the normal-weight group. Similar findings have been reported in previous studies [4,12]. The frequency of elevated non-HDL-C was three times higher in participants with WHtR ≥ 0.5 than in their counterparts with WHtR < 0.5. Our finding regarding the positive association of high BMI and WHtR ≥ 0.5 with high serum non-HDL-C level may be explained by the influence of obesity on cholesteryl ester transfer protein (CETP) activity. General obesity (as measured by high BMI) and central obesity (as measured by WHtR ≥ 0.5) are known to induce increase in CETP activity, resulting in hypercholesterolaemia and decrease in serum high-density lipoprotein cholesterol (HDL-C) levels (21,22). Thus, accounting for the higher mean serum non-HDL-C levels observed among overweight/obese adolescents in the present study. The results of a study in Brazil suggest that waist-to-height ratio more accurately predicted dyslipidaemia than BMI (23).

The overall mean serum concentration of non-HDL-C was higher in overweight/obese than normal weight participants. A similar finding has been reported in previous studies (5,12). The mean serum non-HDL-C concentration was higher in girls than boys. This finding is consistent with the report of several studies among adolescents in USA, China, Turkey and India (12,13,24,25). The observed gender difference in serum profile of non-HDL-C may be explained by the established age- and puberty-related dynamic changes in serum lipid levels. Goff et al [26], reported that mean serum total cholesterol levels tended to be steady during prepubertal period, dropped during puberty in both sexes, with the drop being more profound in boys, and then rise again during in late adolescence. Similarly, in a study in Turkey, serum total cholesterol levels showed a more profound decline in boys starting from the age of 9 years until the age of 15 years and then began to rise at the age of 17 years (21). Considering that serum non-HDL-C level is derived from the difference between total cholesterol and HDL-C, a more profound decline in serum total cholesterol level in boys will result in a relatively lower serum non-HDL-C level in boys than girls. Thus, accounting for the higher serum non-HDL-C levels in girls.

In conclusion, the risk of developing a significantly elevated non-HDL-C level was higher in overweight/obese than normal-weight adolescents. It is advocated that non-HDL-C level should be estimated routinely in serum lipid panels in children and adolescents, particularly those with a high body mass index.
**Strengths and weaknesses of the study**

The strength of the study is that the subjects and controls were derived from a single well defined source population. In addition, matching increased the statistical precision of estimates, thereby allowing smaller sample size. The weakness of the study was that the study population was derived from a single school, making it possible that the sample was not representative of adolescents in the source population. Despite this limitation, it provided baseline epidemiological data (the first among Nigerian adolescents) on the subject and could serve as an initial point for future study.

**Conflict of interest**

We have no conflict of interest in this study.
References

1. Sniderman AD, Hogue JC, Bergeron J, Gagué C, Couture P. Non HDL cholesterol and apolipoprotein B in dyslipidaemia. Clin Sci 2008;114:149-155.

2. Srinivasan SR, Frointi MG, Xu J, Berenson GS. Utility of childhood non-high density lipoprotein cholesterol levels in predicting adult dyslipidemia and other cardiovascular risks: The Bogalusa Heart Study. Pediatrics 2006;118:201-206.

3. Zhu HF, Liang L, Wang CL, Fu JF. Triglyceride and non-high-density lipoprotein cholesterol as predictors of cardiovascular disease risk factor in Chinese Han children. Indian Pediatr 2013;50:394-398.

4. Srinivasan SR, Myers I, Berenson GS. Distribution and correlates of non-high-density lipoprotein cholesterol in children: The Bogalusa Study. Pediatrics 2002;110(3):e29.

5. Sugiuara O, Okada T, Yamauchi K, Murata M. The influence of underweight or obesity to lipid profiles in Japanese children. J Child Health 2015;74:656-661.

6. Corey KE, Lai M, Gelrud LG, Misdraji J, Barlow LL, Zheng H, et al. Non-high-density lipoprotein cholesterol as a biomarker of nonalcoholic steatohepatitis. Clin Gastroenterol Hepatol 2012;10:651-656.

7. Li C, Ford ES, McBrirde PE, Kivotoroicz PO, Crindrille BW, Gilding SS. Non-high-density lipoprotein cholesterol concentration is associated with metabolic syndrome among US youths aged 12-19 years. J Pediatr 2011;158:201-207.

8. Sniderman AD, Williams K, Contois JH, Monroe HM, McQueen MJ, de Graaf J, Furberg CD. A meta-analysis of low density lipoprotein cholesterol, non high density lipoprotein cholesterol and apolipoprotein B as markers of cardiovascular risk. Circ Cardiovasc Qual Outcomes 2011;4:337-345.

9. Miller M, Cannon CP, Murphy SA, Qin J, Ray KK, Braunwald E. Impact of triglyceride levels beyond low-density lipoprotein cholesterol after acute coronary syndrome in the PROVE IT-TIMI 22 Trial. J Am Coll Cardiol 2008;51:724-730.

10. Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents; summary report. Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents; National Heart, Lung and Blood Institute. Pediatrics 2011;128(Suppl 5):S213-S256.

11. Abe Y. Difference in serum non-high-density lipoprotein cholesterol levels in terms of sex, age, and physique in children and adolescents. J Atheroscler Thromb 2016;23:1311-1312 [Editorial].

12. Dai S, Yang Q, Yuan K, Loutalot F, Fang J, Daniels SR, Hong Y. Non-high-density lipoprotein cholesterol: Distribution and prevalence of high serum levels in children and adolescents: United States National Health and Nutrition Examination Surveys, 2005-2010. J Pediatr 2014;164(2):247-253.

13. Fang TL, Liang L, Fu JF, Gong CX, Xiong GC, Liu GL, Luo FH, Cheng SK. Levels of non-high density lipoprotein cholesterol and its related factors in Chinese Han students. HK J Pediatr (new series) 2013;18:210-216.

14. Bao W, Srinivasan SR, Wattigney WA, Berenson GS. Persistence of multiple cardiovascular risk clustering related to syndrome X from childhood to young adulthood. Arch Intern Med 1994;154:1842-1847.

15. Marfell-Jones M, Olds T, Stewart A, Carter L. International Standards for Anthropometric Assessment, 2nd edition. The International Society for the Advancement of Kinanthropometry, Australia, 2006.

16. McCarthy HD, Ashwell M. A study of central fatness using waist-to-height ratio in UK children and adolescents over two decades supports the simple message – ‘keep your waist circumference to less than half your height’. Int J Obes (Lond) 2006;30:988-992.

17. Sass AE, Kaplan DW. Adolescence. In: Hay WW Jr, Levin MJ, Deterding RR, Alzuzg MJ (eds). Current Diagnosis and Treatment: Pediatrics 22nd edition, McGraw Hill Education Publishers, New York, 2014:117-157.

18. Taberi F, Chahkandi T, Kazemi T, Bijari B, Zardast M, Namakian K. Lipid profiles and prevalence of dyslipidemia in Eastern Iranian adolescents, Birjand, 2012. Iran J Med Sci 2015;40(4):341-348.

19. Bilaloni MD, Salas R, De la Garza YE, Villureal JZ, Sureda A, Tur JA. Serum lipid profile, prevalence of dyslipidemia and associated risk factors among northern Mexican adolescents. J Pediatr Gastroenterol Nutr 2016;63:544-549.

20. Al-Daghari NM, Alijohani NJ, Al-Arast OS, Al-Saleh Y, Wani K, Alnaimi AM, Alfaawz H, Al-Afian ASM, Al-Mam S, Chrousos GP, Alokail MS. Non-high-density lipoprotein cholesterol and other lipid indices vs elevated glucose risk in Arab adolescents. J Clin Lipidol 2015;9(3):35-41.

21. Asayama K, Hayachibe H, Dobashi K, Uchida N, Nakane T, Kodera K, Shirahata A. Increased serum cholesterol ester transfer protein in obese children. Obesity Res 2002;10(6):439-446.

22. Mietinen TA, Gylling H. Cholesterol absorption efficacy and sterol metabolism in obesity. Atherosclerosis 2000;153:241-248.

23. de Quadros TMB, Godina AP, da Silva LR. Predictive capacity of anthropometric indicators for dyslipidemia screening in childhood and adolescents. J Pediatr (Rio J) 2015;91(5):455-463.

24. Ucar B, Killic Z, Dinleyici EC, Colak O, Gunes E. Serum lipid profiles including non-high density lipoprotein cholesterol levels in Turkish school children. Anadolu Kardiylol Derg 2007;7:415-420.
Nijagun N, Vani HN, Niranjan HS, Suresh TN, Sanjeeva GN. Study of lipid profile and prevalence of dyslipidemia in adolescent school children from Karnataka. *Int J Pharm Biol Sci* 2015;5(1):79-85.

Goff DC Jr, Donker GA, Rgan JA Jr, Adikins AT, Killinger RP, Candill JW Jr, et al. Cholesterol screening in pediatric practice. *Pediatrics* 1991;88:250-258.

**BMI =** Body mass index; **WC =** Waist circumference; **HC =** Hip circumference

**TC =** Total cholesterol; **HDL-C =** High-density lipoprotein cholesterol; **TG =** Triglyceride; **LDL-C =** Low-density lipoprotein cholesterol.

### Table 1. Clinical and biochemical characteristics of the subjects and controls according to gender

| Clinical Characteristics | Subjects Male | Controls Male | t-statistic (p-value) | Subjects Female | Controls Female | t-statistic (p-value) |
|--------------------------|---------------|---------------|-----------------------|-----------------|-----------------|-----------------------|
| Mean weight (kg)         | 52.3±6.4      | 39.1±7.8      | 9.158 (< 0.001)       | 40.6±5.2        | 36.3±8.4        | 3.047 (< 0.01)        |
| Mean height (cm)         | 149.3±6.8     | 142.4±6.6     | 5.097 (< 0.01)        | 146.8±7.3       | 140.3±5.6       | 4.945 (< 0.01)        |
| Mean BMI (kg/m²)         | 25.32±7.2     | 16.8±2.15     | 9.800 (< 0.001)       | 27.7±3.3        | 17.5±3.5        | 16.300 (< 0.01)       |
| Mean WC (cm)             | 83.2±4.9      | 68.8±4.4      | 15.777 (< 0.001)      | 88.9±5.2        | 69.1±6.7        | 16.342 (< 0.001)      |
| Mean HC (cm)             | 90.3±10.6     | 70.8±8.8      | 9.908 (< 0.001)       | 102.3±8.7       | 72.5±8.6        | 17.052 (< 0.001)      |
| Mean WC/HC ratio         | 0.87±0.08     | 0.85±0.05     | 3.710 (< 0.001)       | 0.9±0.05        | 0.80±0.06       | 5.378 (< 0.001)       |
| Mean TC (mg/dl)          | 136.3±26.6    | 124.2±23.8    | 1.652 (> 0.05)        | 141.0±25.8      | 133.2±20.9      | 1.243 (> 0.05)        |
| Mean HDL-C (mg/dl)       | 37.0±5.2      | 37.2±4.6      | 0.132 (> 0.05)        | 38.1±6.1        | 37.1±5.3        | 0.655 (> 0.05)        |
| Mean TG (mg/dl)          | 101.8±27.2    | 90.3±25.6     | 1.414 (> 0.05)        | 102.5±23.7      | 95.5±22.7       | 1.129 (> 0.05)        |
| Mean LDL-C (mg/dl)       | 68.5±24.2     | 65.7±25.8     | 0.363 (> 0.05)        | 76.7±20.2       | 73.1±18.8       | 0.690 (> 0.05)        |

### Table 2: Prevalence of acceptable (normal), borderline and abnormal (high) non-high density lipoprotein cholesterol levels in overweight/obese and normal weight participants.

| Non-high density lipoprotein cholesterol (mg/dl) | Overweight/Obese | Normal-Weight | Z-statistic (p-value) |
|------------------------------------------------|------------------|--------------|-----------------------|
| Acceptable (<120)                               | 36(73.5)         | 45(91.8)     | 2.701 (< 0.01)        |
| Borderline (120-144)                             | 12(24.5)         | 4(8.2)       | 2.237 (< 0.05)        |
| Abnormal (≥145)                                 | 1(2.1)           | 0(0.0)       | 0.207 (> 0.05)        |
| Total                                           | 49(100.0)        | 49(100.0)    |                       |

Borderline plus abnormal non – HDL- C: Subjects vs controls Z- statistics 2.666(p Value <0.01)
### Table 3: Prevalence of acceptable (normal), borderline and abnormal non-high density lipoprotein cholesterol levels in subjects and controls according to waist-height ratio.

| Parameter                        | WHtR ≥ 0.5 | WHtR < 0.5 | Z-statistic (p-value) |
|----------------------------------|------------|------------|-----------------------|
| Acceptable (<120)               | 36(73.5)   | 45(91.8)   | 2.701 (≤ 0.01)        |
| Borderline (120-144)            | 12(24.5)   | 4(8.2)     | 2.237 (≤ 0.05)        |
| Abnormal (≥145)                 | 1(2.1)     | 0(0.0)     | 0.207 (> 0.05)        |
| Total                           | 49(100.0)  | 49(100.0)  |                       |

WHtR = Waist-to-height ratio

### Table 4: Mean serum non-high-density lipoprotein concentrations and prevalence of elevated levels in overweight/obese and normal-weight participants, according to gender.

| Parameter                        | Overweight/Obese | Normal weight | Statistical analysis |
|----------------------------------|-------------------|---------------|----------------------|
| Mean±SD serum non-HDL-C         |                   |               | t-test               |
| concentration                   |                   |               |                      |
| Males(21)a                      | 95.0±25.2         | 91.1±24.7     |                      |
| Females(28)b                    | 106.1±21.9        | 95.3±22.5     | a vs b: 1.613; p>0.05 |
| Both sexes(49)                  | 101.4±23.3        | 93.6±24.8     | Z-test               |
| Prevalence (%) of high non-HDL-C concentration |                   |               |                      |
| Males(21)c                      | 3(14.3)           | 1(4.8)        | c vs d: 1.946; p>0.05 |
| Females(28)d                    | 10(37.5)          | 3(10.7)       |                      |
| Both sexes(49)                  | 13(26.5)          | 4(8.2)        |                      |
Introduction
Autonomous production of parathyroid hormone results in primary hyperparathyroidism. Sri Lankan studies analyzing cases of hyperparathyroidism are scarce.

Objective
To describe clinical, biochemical, radiological and pathological characteristics of patients with primary hyperparathyroidism undergoing surgical intervention in two tertiary care hospitals in Sri Lanka.

Design
Data was collected from the available records of all patients who underwent surgery for primary hyperparathyroidism at Teaching Hospital, Kandy and General Hospital, Badulla from 2016.10.27 to date. A total of ten patients with primary hyperparathyroidism were enrolled for analysis. Out of the ten patients, six were females. Ages ranged from 23 to 65 years (Mean 44.2). Mode of presentation was fragility fractures in four patients, renal calculi in five patients and acute pancreatitis in one patient. Bone complications (fractures, osteoporosis) were seen in four patients and renal complications (renal calculi, nephrolithiasis) were seen in six patients. Pre-operative total serum calcium levels ranged from 10.52 to 14.16 mg/dl (mean-12.6). Pre-operative serum phosphate levels ranged from 0.74 to 1.07 mmol/L (mean -0.868).

X-ray evidence of skeletal changes of hands were seen in 6 patients (60%), of which 4 showed bony deformities and 2 showed pathological fractures. Renal calculi or nephrolithiasis was detected by X-ray in five out of six patients and ultrasonically in all 6 patients. Ultrasound scans revealed a parathyroid adenoma in 8 patients (80%), while 2 were negative. Technetium-99-methoxyisonitrile (99mTc-Sestamibi) scans were performed in 4 patients and three had positive images. Histological analysis revealed 8 patients with single parathyroid adenoma, 1 with parathyroid carcinoma, and one patient with four gland hyperplasia.

Discussion and Conclusions
All patients had symptoms at the time of diagnosis. Ultrasound scan was a useful tool for the localization.
**Methodology**

Prospectively data was collected from patients’ records of all primary hyperparathyroid patients who underwent surgery at Teaching Hospital, Kandy and General Hospital, Badulla from 2016.10.27 to date. Patients’ clinical features, biochemical and imaging findings, surgical procedures and histological findings were collected.

**Statistical Analysis**

Statistical analysis was done with Epiinfo version:7.2.2.6. Means and range of age, total corrected Calcium, phosphate, serum PTH and parathyroid gland size were done. Sensitivity and specificity of the ultrasound scan imaging of the parathyroid gland was analysed.

**Results**

Out of the 10 patients, 6 were females. Ages ranged from 23 to 65 years (Mean-44.2). Triggers for the evaluation for primary hyperparathyroidism were, fragility fractures or bony deformities in 4 patients, renal calculi in 5 patients and acute pancreatitis in one patient (Table 1). The initial total serum calcium level ranged from 10.52 to 14.16 mg/dl (mean-12.6) and serum phosphate level ranged from 0.74 to 1.07 mmol/l (mean - 0.868). (Laboratory Calcium and phosphate reference ranges were 8.4mg/dl to 10.2mg/dl and 0.81 to 1.45mmol/l respectively). Average parathyroid hormone level (Normal PTH level -6-80) was 1306pg/ml for parathyroid adenomas (ranged from 340 to 1849), 1082 pg/ml for the only parathyroid carcinoma and 212pg/ml for multi gland disease(Table 2). Dual-energy X-ray absorptiometry (DXA) scans were performed in 5 patients, of which 4 had osteoporosis (mean bone mineral density BMD -0.498) and one had osteopaenia (BMD -0.826).

| Triggers for the evaluation                  | Number of patients |
|---------------------------------------------|--------------------|
| Fragility fracture or bony deformity        | 4                  |
| Renal calculi                               | 5                  |
| Acute pancreatitis                          | 1                  |
| Total                                       | 10                 |

**Table 1. Mode of presentation for primary hyperparathyroidism**

|                          | Mean       | Range       |
|--------------------------|------------|-------------|
| Total corrected Ca       | 12.64mg/dl | 10.52-14.16 |
| Phosphate                | 0.87mmol/l | 0.74-1.07   |
| PTH (Parathyroid adenoma)| 1306pg/ml  | 340-1849    |
| PTH (Parathyroid carcinoma)| 1082pg/ml  |             |
| PTH (Parathyroid hyperplasia)| 212pg/ml  |             |

All patients underwent ultrasound scan of the parathyroid glands and it identified a parathyroid tumour in 8 patients (Table 3). Although in one patient’ ultrasound was suggestive of a thyroid nodule, subsequent CT scan reported the corresponding lesion as a possible parathyroid tumor. Finally, it turned out to be a parathyroid carcinoma.

The second person who had a negative ultrasound scan underwent 99m Tc-Sestamibi and it was also negative. Four patients underwent 99m Tc-Sestamibi including the above mentioned patient. In the remaining three patients it identified a parathyroid tumour.
During surgery the location of the enlarged parathyroid gland was concordant with the ultrasound scan, CT scan and Tc99m Sestamibi findings. In the patient who had negative ultrasound scan and 99m Tc Sestamibi, only 3 parathyroid glands were identified during surgery. Patients who had positive localization with ultrasound scan and technetium scan had a parathyroid adenoma at surgery and they were cured postoperatively. The one who had negative ultrasound with localization by CT scan had parathyroid carcinoma without evidence of metastasis. The one who had negative ultrasound scan and sestamibi scan, only three glands were identified and removed but he was not cured postoperatively. Finally the remaining gland was localized by parathyroid venous sampling. Repeat surgery detected remaining right upper parathyroid gland.

Histological evaluation of the surgical specimen showed single parathyroid adenoma in eight patients, parathyroid carcinoma in one and four gland hyperplasia in one who had initial negative ultrasound and 99m Tc-Sestamibi.

Discussion

Clinical and biochemical features of patients who underwent parathyroid surgery presented in recent Western literature were as follows: majority of the cases occurred over the age of 50-65 years and majority of them were women(3),(4). Asymptomatic primary hyperparathyroidism was seen in 80% of cases. Average serum Calcium was 10.8±0.1 mg/dl, Serum PTH level 144 pg/ml, renal stones accounted for 15% of cases, average lumbar BMD Z score -0.8 ±0.2and sensitivity and specificity of USS imaging was 42-82% and approximately 90% respectively(1),(5). Average weight of the parathyroid adenoma was 1g (normal gland weighs 50mg)(6). However, in our cohort all patients were symptomatic. It had higher mean Calcium value and Serum PTH value. Out of the patients who ever underwent DXA (N=5),four had osteoporosis and one had osteopenia. Sensitivity and specificity of USS on localization were 89% and 100% respectively.

An article published in Lancet in 1980 describes changing pattern of presentation over time. It shows that in earlier studies majority of them were symptomatic at the time of presentation. Symptoms were mainly attributed to renal stones, nephrocalcinosis and bone disease as in our series. However during the 1980s, majority of the patients (57%)were asymptomatic probably due to routine measurement of Calcium(7).

Our data suggests that the Sri Lankan picture of patients who presented with hyperparathyroidism is similar to what was seen in the Western hemisphere before 1980. There are several possibilities for this scenario. Only recently serum Calcium became widely available in Sri Lanka and PTH assays are still limited to a few centres. Although facilities for Serum Calcium measurement is freely available now, it is not routinely requested by clinicians. Lack of awareness among health care professionals regarding this common endocrinological disorder is also an important factor. As a result all patients were symptomatic at presentation and none had normocalcaemic hyperparathyroidism despite vitamin D deficiency being prevalent in this country(8).A population based study done at Rochester, Minnesota in between 1965-1992 suggested that routine measurement of serum Calcium led to a sharp increase in the incidence of

| Patient No. | USS Neck | CECT | Tc99m Sestamibi scan | Surgery | Histology |
|-------------|----------|------|---------------------|---------|----------|
| 1-8         | Parathyroid adenoma | Not done | Parathyroid adenoma(n=3) | Selective parathyroidectomy | Parathyroid adenoma |
| 9           | Only thyroid nodule seen | Parathyroid adenoma corresponding to USS lesion | Not done | Selective parathyroidectomy | Parathyroid carcinoma |
| 10          | No abnormality detected | Not done | No abnormality detected | 2 ½ parathyroid glands removed | Parathyroid hyperplasia |

Table 3. Imaging findings in patients with primary hyperparathyroidism
primary hyperparathyroidism\textsuperscript{(2)}. So in our country also there should be a rise in incidence in upcoming years due to increased availability of Serum Calcium measurement. We are planning to recruit and follow-up more primary hyperparathyroid patients during upcoming years.

Ultrasound scan had more sensitivity in our series compared to the data in Western literature. Although there is no explanation for this, late presentation among our patients may be a reasonable postulation that needs to be investigated further.

**Conclusion**

Although majority of the patients with primary hyperparathyroidism in the Western world are asymptomatic at the time of diagnosis, in our small case series all had symptoms at the time of presentation. It suggests that more awareness is required among medical practitioners to diagnose this condition early.

Ultrasound scan is a cheap, readily available and less invasive investigation in localizing parathyroid adenoma in resource poor settings. In the hands of good sonographer it has a high positive predictive value. When an imaging modality does not localizes the tumour, multi gland disease should be suspected.
References:

1. Silverberg SJ, Bilezikian JP. Evaluation and management of primary hyperparathyroidism. *J Clin Endocrinol Metab* [Internet]. 1996 Jun 1;81(6):2036–40. Available from: http://dx.doi.org/10.1210/jcem.81.6.8964825

2. Wermers RA, Khosla S, Atkinson EJ, Hodgson SF, O’Fallon WM, Melton LJ 3rd. The rise and fall of primary hyperparathyroidism: a population-based study in Rochester, Minnesota, 1965-1992. *Ann Intern Med*. 1997 Mar;126(6):433–40.

3. Marcocci C, Cetani F. Clinical practice. Primary hyperparathyroidism. *N Engl J Med*. 2011 Dec;365(25):2389–97.

4. Clifford JR. Pathogenesis and etiology of primary hyperparathyroidism. In: In: UpToDate, Post TW (Ed), UpToDate, Waltham, MA (Accessed on December 26, 2018).

5. John PB. Primary Hyperparathyroidism. In. www.endotext.org, version of January/D12/2017, (25.01.2018), published by MDTEXT.COM,INC, South Dartmouth,MA 02748.

6. Wienke JA, Smith A. Parathyroid adenoma. Head Neck Pathol [Internet]. 2008/10/22. 2008 Dec;2(4):305–8. Available from: https://www.ncbi.nlm.nih.gov/pubmed/20614300

7. Mundy GR, Cove DH, Fisken R. Primary hyperparathyroidism: changes in the pattern of clinical presentation. *Lancet* (London, England). 1980 Jun;1(8182):1317–20.

8. Meyer HE, Holvik K, Lofthus CM, Tennakoon SUB. Vitamin D status in Sri Lankans living in Sri Lanka and Norway. *Br J Nutr*. 2008 May;99(5):941–4.
Case Report

Recurrent haematuria; a rare presentation of 46XX Congenital adrenal hyperplasia presenting late and reared as males – two cases

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Abstract

Congenital adrenal hyperplasia (CAH) is a group of inherited autosomal recessive disorders characterized by a defect in enzymes involved in biosynthesis of cortisol, aldosterone or both. We report two patients presented with recurrent haematuria with two different forms of CAH who presented late and reared as males. We describe the challenges posed on managing them and how the quality of life will be improved by offering hormonal and surgical remedies without changing or reassigning the gender to females.

Key words: Congenital adrenal hyperplasia, gender assignment, recurrent haematuria, 21-hydroxylase, 11-beta hydroxylase

Introduction

Congenital adrenal hyperplasia (CAH) is a group of inherited autosomal recessive disorders characterized by a defect in enzymes involved in biosynthesis of cortisol, aldosterone or both (1). The commonest type is 21-hydroxylase (21-OH) deficiency accounting for 90 to 95%. The second commonest type is 11-beta hydroxylase deficiency (6-8%) (2). In CAH loss of negative feedback from cortisol results in ACTH hypersecretion, and adrenocortical hyperplasia (3). Accumulation of steroid precursors above the enzyme deficiency shunted into androgen synthesis pathway will result in androgen excess (Figure 1). The clinical presentation depends on severity of mutation and this vary from neonatal salt wasting, virilisation to non-classic CAH in adulthood. The degree of virilisation in a female CAH (46XX) also vary from nearly female looking external genitalia to completely male looking ones with prader stages 2 – 5. In these cases, if the diagnosis is made early during infancy, gender assignment as female is not debatable. When presented later and already reared as males and presenting with late complications such as haematuria, gender re-assignment and management is highly challenging.

The following describes two adults with 2 different forms of CAH presenting very late with recurrent haematuria and the challenges posed on managing them.

Patient S

A 36-year-old man presented to the surgical clinic with intermittent painless hematuria for last 2 years. He was referred to Endocrinology unit for short stature and gynaecomastia. His childhood and family history was unavailable. Until this admission, no expert medical help had been sought for his short stature. He was single and working as a manual labourer. His height was 137.5cm and weighed 46kg with body mass index of 26 kg/m2. He had bilateral breast enlargement with no male pattern body hair. Genital examination showed tanner 3 staging for pubic hair and hypospadias with meatus at mid shaft of phallus. Testes were not palpable in the under-developed scrotal sac (figure 1). His blood pressure was 120/80 mmHg and rest of the examinations were unremarkable. His karyotype was 46XX. He had elevated 17 hydroxy-progesterone (17 OHP) with slightly elevated testosterone. Although his basal cortisol was normal, the level after ACTH stimulation was suboptimal. Laboratory tests are described in table 1. Imaging with CT showed enlarged left adrenal gland (3.2x3.3cm), uterus and ovaries. The diagnosis of CAH (21 hydroxylase deficiency) was made and this complexity and consequences of the pathology could have on the patient’s life were explained. Knowing the fact that his fertility will not be possible as a male, he decided to keep his sex allocation as male. He underwent surgical removal
of the uterus, ovaries, upper third of the vagina and fallopian tubes under steroid cover (figure 2). Bilateral mastectomy was planned and the possibility of staged male genitoplasty comprising of chordee correction, male urethroplasty and insertion of artificial testicular prosthesis in the labioscrotal sac was discussed with the patient. He was advised on the need for steroid cover during severe illness and surgery and steroid alert card was given. Monthly testosterone replacement was started.

Patient informed consent and permission to use these views was granted in accordance with recognised guidelines on the ethics of such photography.

Patient RA 23 years old man was transferred from a local hospital with the history of abdominal pain, haematuria and high blood pressure. He was the third child of non-consanguineous parents from a very low socio economic background. He had been admitted to the local hospital at the age of fifteen with headache and haematuria. At that time he was treated for hypertension and followed up for few months. Three years ago he was re admitted with painful genitalia and haematuria associated with clots especially at the end of the micturition. This time he was transferred to National hospital of Sri Lanka for further evaluation for short stature & haematuria. While investigating he dropped out from follow-up. He had 4 healthy siblings. He was average in school performance; studied up to grade 9. But he dropped out from school due to bullying related to his short stature. At admission
to local hospital he was hypertensive with a blood pressure of 240/120 mmHg. He was very short with a height of 130 cm and weighed 36.2 kg (body mass index of 21.42 kg/m²). There was no cutaneous hyperpigmentation. He had male pattern facial and body hair. His genitals were Tanner staging 4 for pubic hair, with phallus length of 4 cm. He had distal hypospadias with sub-coronal meatus. Scrotum was not well formed and testes were not palpable (figure 4). He had hypertensive retinopathy changes with silver wiring.

Karyotype was 46XX and was negative for SRY gene. Biochemistry and endocrine investigations are shown in table 1. He had elevated 17 OHP and testosterone levels. His basal as well as ACTH stimulated cortisol levels were very low indicating adrenal insufficiency. Imaging with ultrasound scan showed rudimentary uterus and MRI showed enlarged adrenals with cystic areas, uterus with bilateral small ovaries.

The diagnosis of CAH (11 beta hydroxylase deficiency) was made based on available clinical and biochemical findings. The levels of 11-deoxycortisol and ACTH were not measured due to availability problems in the region. Similar to previous patient, he also decided to keep his sex allocation as male. Surgical removal of the uterus, ovaries, upper third of the vagina and fallopian tubes and bilateral mastectomy was planned and the possibility of staged male genitoplasty comprising of chordee correction, male urethroplasty and insertion of artificial testicular prosthesis in the labioscrotal sac was discussed with the patient. He was advised on the need for long term therapy with steroid and steroid alert card was given. Monthly testosterone replacement was started along with hydrocortisone and anti-hypertensives.

![Figure 4](image)

Table 1. Laboratory investigations of the patients with congenital adrenal hyperplasia

| Test                      | Patient S | Patient R | Reference Value |
|---------------------------|-----------|-----------|-----------------|
| Total testosterone (ng/ml)| 1.87      | 3.8       | 0.3 – 1.2       |
| 17 OHP – basal            | 14        | 12.8      | < 5             |
| 17 OHP, 60 min post synacthen (nmol/L) | 93.06 | 95 | < 30 |
| DHEA-sulphate (µg/ml)    | 4.2       | 5.4       | 0.9-3.6         |
| LH (mIU/L)                | 12.2      | 3.72      | 2-20            |
FSH (mIU/L) | 9.72 | 5.4 | 2-20
Free T4 (ng/dl) | 1 | 1.7 | 0.9-1.7
TSH (µIU/ml) | 3.52 | 3.3 | 0.3-4.20
Cortisol (nmol/l) | 252 | 60 | 123-626
Cortisol – 30 min post synacthen (nmol/l) | 382 | 64.5 | >550 nmol/l
FBS (nmol/l) | 4.6 | 4.3 | 3.9-5.5
Serum K+ (mmol/l) | 4.2 | 3.7 | 3.5-5.5
Serum Na+ (mmol/l) | 4.6 | 4.3 | 3.9-5.5
Serum creatinine (mg/dl) | 2.21 | 2.18 | 2.1-2.55
Serum calcium (mmol/l) | 0.87 | 0.9 | 0.6-1.2
Albumin (g/L) | 1.137 | 135 | 135-148

Discussion

Gender identity is the sense of belonging that one feels for a particular sex psychologically and socially independent of one's biological sex. Gender assignment become very complex if the genitalia are highly virilised. Apart from karyotype, sociocultural influences and parental expectations play a significant role in this. There is scarcity of data on gender identity among females with CAH (4). Some studies showed among 46XX CAH, only a minority developed gender dissatisfaction when reared as females, but there are also reports of successful male gender assignments (5, 6).

These two patients have been raised as boys since birth and they are satisfied with their male gender identity. Although both of them were very short and had ambiguous genitalia, they did not seek medical attention until very late. Cyclical haematuria secondary to uterine bleeding was the main reason for them to seek medical advice. The reason for delayed presentation could be the family's desire for a male child in our sociocultural background or ignorance that early treatment is required. The diagnosis of CAH was made very late in these two patients when they have already had a firm male gender identity and the society and family has accepted them as males. Therefore we offered individualized treatment considering their medical, social and psychological wellbeing.

Management of classic form of CAH includes replacement of cortisol and aldosterone to control excess androgen symptoms. Treatment of adult patients with CAH remains controversial due to lack of randomized controlled trials comparing treatment regimens (7). The Endocrine society guidelines recommend glucocorticoid treatment for adult CAH only individuals with a significant degree of hyperandrogenism or those who desire fertility (8). Hydrocortisone is the drug of choice in children, but in adults, long acting glucocorticoids such as prednisolone and dexamethasone can be used without concern about growth inhibition. Careful individualized therapy to optimize psychological, reproductive and sexual and bone health, avoiding steroid related side effects will benefit adults with CAH.

Although the fertility will not be possible as males in our patients, they could function as normal males with regard to marriage, relationship and occupation. There were few favourable points to these two patients; male gender identity and partial male external body habitus. Moreover they need not to face the legal issues of changes in name and sex on birth certificates and identity cards.

Shilpa Sharma and Devendra Gupta have described seven children with CAH presented late with severe virilisation and reared as males. In this series patients underwent male genitoplasty and surgical removal of female adnexa. Over the mean follow-up duration of 9.2 years, majority were well adjusted and had good surgical and social outcome, but one had recurrent urinary tract infection and poor social adjustment (6).

Although CAH patients who presented late have different disease characteristics, the major sex reassignment surgical interventions in the female to male transsexual patients can be utilized for this patients. The main interventions are subcutaneous mastectomy combined with hysterectomy and ovariectomy and male genitoplasty. The interventions may differ for each patients depending on their degree of virilisation. Creation of a male chest by means of a subcutaneous mastectomy allows the patient to live more easily in the male role. Removal of the female adnexa; uterus, ovaries, fallopian tube and upper vagina will avoid other complications such as uterine bleeding presenting as haematuria like in our two patients. Staged genitoplasty generally comprising of chordee correction, male urethroplasty and scrotoplasty. At a later stage, a testicular prostheses can be inserted (9). They need hormonal treatment with testosterone replacement with monitoring of hematocrit and lipid profile.
Conclusion

Gender assignment in patients with ambiguous genitalia is a complex process especially if there is discordance between genetic and phenotypic characteristics. In patients with 46XX CAH, if the diagnosis is made in early infancy, the sex of rearing is female even the genitalia is fully virilised. However this will be complicated in delayed patients with the concurrent development of masculine identity.

Therefore early diagnosis by improving awareness among general physicians is important. In delayed or inadvertent late presentations, the management should be individualized. The quality of life will be improved by offering hormonal and surgical remedies without changing or reassigning the gender to females. This two patients were comfortable with the surgical outcome and willing to undergo further planned surgical interventions.

References

1. Merke DP, Bohnstein SR, Avila NA, Chrousos GP. NIH Conference. Future directions in the study and management of congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Ann Intern Med. 2002, 136 (4): 320-334.
2. Zachmann M, Tassinari D, Prader A. Clinical and biochemical variability of congenital adrenal hyperplasia due to 11-beta-hydroxylase deficiency. A study of 25 patients. J Clin Endocrinol Metab. 1983;56(2):222–229.
3. Oxford handbook for diabetes endocrinology and metabolism, third edition.
4. Kukreti P, Kandpal M, Jiloha RC. Mistaken gender identity in non-classical congenital adrenal hyperplasia. Indian Journal of Psychiatry. 2014;56(2):182-184. doi:10.4103/0019-5545.130504.
5. Gangaher A, Jyotsna VP, Chauhan V, John J, Mehta M. Gender of rearing and psychosocial aspect in 46 XX congenital adrenal hyperplasia. Indian Journal of Endocrinology and Metabolism. 2016;20(6):870-877. doi:10.4103/2230-8210.192922.
6. Shilpa Sharma and Devendra K. Gupta. Male genitoplasty for 46 XX congenital adrenal hyperplasia patients presenting late and reared as males. Indian J Endocrinol Metab. 2012 Nov-Dec; 16(6): 935–938. doi: 10.4103/2230-8210.102994
7. Merke DP. Approach to the Adult with Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency. The Journal of Clinical Endocrinology and Metabolism. 2008;93(3):653-660. doi:10.1210/jc.2007-2417.
8. Speiser PW, Azziz R, Baskin LS, et al. A Summary of the Endocrine Society Clinical Practice Guidelines on Congenital Adrenal Hyperplasia due to Steroid 21-Hydroxylase Deficiency. International Journal of Pediatric Endocrinology. 2010;2010:494173. doi:10.1155/2010/494173.
9. Monstrey SJ, Ceulemans P, Hoebeke P. Sex Reassignment Surgery in the Female-to-Male Transsexual. Seminars in Plastic Surgery. 2011;25(3):229-244. doi:10.1055/s-0031-1281493.
Case Report

Pituitary Metastasis: Central Diabetes Insipidus unmasked by Corticosteroids – Case Series and Review of Literature

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Key words: Pituitary metastasis, Central diabetes insipidus, Pan-hypopituitarism, Corticosteroids

Background

Metastasis to the pituitary gland is rare encounter and is more common amongst the elderly population with advanced malignancy. An estimated 1% of all pituitary tumour resections are metastatic. Primary sites that frequently metastasize include breast and lung carcinomas. In the recent decade, advancement in the field of oncology with multiple modalities of therapy has led to prolonged survival of patients with advanced stages of malignancy. Herein, we present three cases and review of literature of pituitary metastasis presenting as central diabetes insipidus (CDI) incidentally unmasked following administration of corticosteroids.

Objective

To establish the common clinical features, establish variations in clinical presentations and natural progression of disease in patients with pituitary metastasis.

Methods

Three cases of central diabetes insipidus unmasked by corticosteroids in pituitary metastasis were presented. A total of 9 other cases with central diabetes insipidus as first clinical manifestation unmasked by corticosteroid published from 2007-2018 were reviewed. Pertinent references were searched using windows remote search model on PubMed. The key words “pituitary metastasis” and “diabetes insipidus” searched in all fields resulted in 161 articles, of which articles of cranial diabetes insipidus as initial presentation without the use of corticosteroids were excluded. Searching “central diabetes unmasked by steroids” retrieved 8 additional references. Searching “metastatic carcinoma of pituitary” retrieved 35 additional references. About 20 new references were identified from the bibliographies of the articles reviewed. Ultimately, we identified a total of 18 references relevant to this research from the search terms. All references were reviewed to retrieve relevant references for this study. Non-English articles were excluded.

Results

A compilation of 9 previously reported cases of central DI unmasked by corticosteroids from 2007 to 2017 along with the present 3 cases were performed (Table 1). There was equal gender prevalence with a mean age of 61 (range 56-80 years old). More than 75% of the cases described here had previously been diagnosed with advanced malignancies of varying primary sites. The remaining 25% presented with varying symptoms of hypopituitarism as the harbinger to the discovery of the primary neoplasm. Amongst the literature review and cases presented, primary malignancies with pituitary metastasis included lung adenocarcinoma (33%), breast carcinoma (25%), nasopharyngeal carcinoma (16%), renal cell carcinoma (8%), hepatocellular carcinoma (8%) and gastric adenocarcinoma (8%). It is noteworthy that two of three present cases identified, were the result of directly infiltration of nasopharyngeal carcinoma to the pituitary gland. There is limited data documenting the prevalence of nasopharyngeal carcinoma with pituitary metastasis within the Asian population.

Conclusion

Central diabetes insipidus unmasked by corticosteroids is a less recognized, potentially lethal but fully reversible complication of pituitary metastasis. Symptoms or signs of central diabetes insipidus should be sought in all patient with advanced malignancies presenting with polyuria and hyponatremia. Prompt restoration of pituitary hormones is warranted in affected patients to allow timely restoration of hormonal balance and preventing endocrine emergencies.
Introduction

Metastasis to the pituitary gland is a rare encounter representing less than 1% of all pituitary lesions. The first reported case of metastasis to the pituitary gland was identified and reported (in German) by Benjamin L. in 1857 discovered during an autopsy of a patient with disseminated melanoma (1, 5, 17). The increased prevalence in detection of pituitary metastasis denotes advancement in oncology treatment and options. These pituitary metastasis in advanced malignancies are most typically identified in the elderly population with diffuse malignancy. The most common primary tumours with metastasis to the pituitary gland are breast, lung and gastrointestinal malignancies. Their scarcity and usually indolent course, as well as the lack of specific clinical and radiological features, impede their differentiation from other more common sellar area lesions, particularly when history of malignancy is absent. Advancement in the field of oncology within the last decade has progressed with enhanced imaging modalities, improved surgical techniques, radical radio- and chemo-therapeutics for the treatment for systemic malignancies. This has led to augmented diagnosis of pituitary metastasis from primary tumours. Despite that, pituitary metastasis remains a challenge for diagnosis and remains poorly recognised and under reported. These pituitary metastasis are often discovered incidentally presenting with central diabetes insipidus having become unmasked after initiation of corticosteroids.

We report 3 clinical cases of pituitary metastasis diagnosed after incidental presentation of central diabetes insipidus after corticosteroid administration. A review of 9 other reported cases from 2007-2017 along with the current three cases were reviewed to establish common clinical features and clinical course of pituitary metastasis.

Case 1

Madam L, 67 year old lady diagnosed three years ago with stage four lung adenocarcinoma with extensive metastases to liver and bone presented to our centre with history of poor oral intake, generalised lethargy and reduced urine output for the past three days. She was admitted and treated as community acquired pneumonia and lymphangitis carcinomatosis where she received a week’s course of intravenous ceftriaxone and 4mg dexamethasone daily. Initial sodium and potassium on admission was 145mmol/L and 4.3mmol/L. However, five days later she developed confusion and polyuria with urine-output up to 4L/day. Initial computed tomography (CT) of the brain showed thickened enhancing pituitary stalk and posterior lobe of the pituitary gland. Magnetic resonance imaging (MRI) showed a lobulated lesion in the right side of the posterior pituitary measuring 0.7 x 1.0 x 0.4cm and a well-defined lesion measuring 0.7 x 0.8 x 0.6cm at the superior aspect of the pituitary infundibulum. Biochemical markers supported the diagnosis of central diabetes insipidus with serum Na 156mmol/L, serum Osmolarity 309mmol/L and urine Osmolarity 145mmol/L. She was treated with subcutaneous desmopressin and intravenous fluids. Further tests demonstrated panhypopituitarism and oral desmopressin, thyroxine and hydrocortisone was initiated. Patient opted for palliative care succumbing to 3 months after admission.

Case 2

Madam S, 56 year old lady diagnosed with stage four nasopharyngeal carcinoma presented to us with symptomatic hyponatraemia in March 2017 having undergone combined chemo-radiotherapy the previous year. She was dehydrated with sodium of 115 mmol/L and potassium 3.9mmol/L. She responded to hydration as sodium increased to 128 mmol/L. However, a week later sodium levels dropped to 119mmol/L despite hydration. CT brain revealed increasing size of primary tumour with intracranial extension involving cavernous sinus, pituitary fossa and left temporal and pontine infiltration. Other investigations support the diagnosis of syndrome of inappropriate antidiuretic hormone secretion and she responded to fluid restriction. In addition, she was diagnosed with hypocortisolism (9am: 26nmol/L) and commenced on oral hydrocortisone 10mg twice daily replacement. Repeat CT staging on revealed disease progression and a pituitary mass of 1.7 x 1.4 x 1.7cm. She was readmitted for chemotherapy but discharged without hydrocortisone in June 2017. In July 2017, she was admitted for her third cycle of chemotherapy with sodium of 123mmol/L, potassium 3.4mmol/L and was restarted on oral hydrocortisone 25mg tds. Unfortunately, she developed thirst and polyuria the same day with increasing sodium trend. Laboratory investigations revealed low urine osmolarity and high serum osmolarity with increasing serum sodium levels supported the diagnosis of central diabetes insipidus and subcutaneous desmopressin was administered. Complete pituitary hormonal panels support panhypopituitarism. She was discharged with hydrocortisone, thyroxine replacement and oral desmopressin but readmitted in September 2017 as her condition deteriorated. Ct brain showed disease progression with enlarging pituitary metastasis. Patient opted for palliative care and succumbed within 1 month.
Case 3

Mr C, a 55 year gentleman was diagnosed with advanced nasopharyngeal carcinoma, undifferentiated type, NOS, TNM: T4 N1 M0, Stage: IVA. He had completed combined chemo-radiotherapy in December 2017. Reassessment CT done in June 2018 revealed residual tumour at left inferior orbital fissure, left optic canal and left pterygopalatine fossa with local infiltration into the left cavernous sinus, pituitary sella and right sphenoid sinus. He was subsequently planned for chemotherapy with Paclitaxel/Carboplatin by the oncologist. He was admitted with symptoms of feeling unwell, vomiting, hypotension and hypoglycaemia. Morning serum cortisol and thyroid function revealed hypopituitarism. He was initiated on oral hydrocortisone and thyroxine replacement and was discharge home. He presented 4 weeks later via emergency with symptoms of polyuria, with inability to compensate and with documented urine output of more than 200mls per hour. Investigations and water deprivation test confirmed the diagnosis of central diabetes insipidus with serum osmolarity of 291, serum sodium of 149 and urine osmolarity 164mmol/L. Further history revealed that the onset of polyuria was soon after initiation of steroids however patient was able to compensate with large volumes of fluid intake, up to 4L/day. He was discharged home with oral desmopressin 0.1mg daily in combination with other pituitary hormone replacement and was discharge home. He presented 4 weeks later with inability to compensate and with documented urine output of more than 200mls per hour. Investigations and water deprivation test confirmed the diagnosis of central diabetes insipidus with serum osmolarity of 291, serum sodium of 149 and urine osmolarity 164mmol/L. Further history revealed that the onset of polyuria was soon after initiation of steroids however patient was able to compensate with large volumes of fluid intake, up to 4L/day. He was discharged home with oral desmopressin 0.1mg daily in combination with other pituitary hormone replacement.

Discussion

The pituitary gland is an uncommon location for metastatic disease, although neoplasms from almost every tissue have been reported to metastasise there. In approximately two third cases of advanced malignancies, the patients were known to have metastatic disease prior to the discovery of pituitary metastasis. On the contrary, a third of patients, pituitary symptoms were the harbinger to the discovery of the primary neoplasm. The most frequent sources of metastases are: breast carcinoma (53% of pituitary metastatic lesions) and lung carcinoma (19%) [1, 5, 17]. Metastatic spread is more common to the pituitary posterior lobe. A review of 201 cases of pituitary metastases demonstrated that the posterior lobe was involved in 84.6% (n = 170), with isolated posterior and anterior lobe lesions seen in 50.8 and 15.4% of cases respectively [2, 3, 5]. Many explanations for this predilection have been proposed. The posterior lobe is perfused directly by the inferior hypophysial arteries, while the anterior lobe is supplied by a portal system around the infundibulum from the superior hypophysial arteries, thus direct haemogenous spread may be more likely to seed to the capillaries of the stalk and posterior lobe. A further contributing factor is the fact the posterior lobe has a larger contact area with adjacent dura, facilitating meningeal spread though the suprasellar cistern [5].

Central diabetes insipidus (DI) is relatively common in pituitary metastasis, present in 42.3% (95% CI 36.2–48.5) of patients at presentation in one pooled study (n = 248) [5]. Moreover, in a patient with known metastatic disease, the development of DI and radiographic evidence of a pituitary mass is strongly suggestive of a pituitary metastasis. DI in the setting of metastasis may be associated with a thickened pituitary stalk in combination with absence of the normal high T1 signal intensity in the posterior lobe [27]. The high incidence of DI in metastatic lesions is consistent with the similarly high incidence of posterior lobe involvement.

Our study described three cases of occult ADH deficiency masked by concurrent ACTH deficiency; only once glucocorticoid replacement therapy had been administered did the symptoms diabetes insipidus appear. This ‘masking’ phenomenon could be due to a multitude of factors, both from ADH-dependent and ADH-independent mechanisms, resulting in impaired renal-free water clearance. Recognition of this phenomenon in patients with adrenal deficiency and risk factors for developing CDI is important in early diagnosis and management of this phenomenon [6].

The reasons for this are complex. Firstly, cortisol induces resistance of the V2 receptor (or at a post-receptor level) to ADH, thus in states of glucocorticoid deficiency, the effects of ADH are amplified [7]. Secondly, Corticotrophin Releasing Hormone (CRH) stimulates ACTH and ADH release, thus glucocorticoid deficiency upregulates CRH and thus ADH release [7, 8]. Lastly, hypocortisolaemia results in renal sodium loss and volume depletion, potent stimulators for increased (but “appropriate”) ADH release. As such, when glucocorticoid deficiency is ameliorated, these compensatory mechanisms fail, and DI ensues. The high rate of DI in our study relative to the literature may be partly explained by our assessment of ADH function both before and after glucocorticoid replacement. Half of our cases had DI on initial assessment, similar to the prevalence in other studies, however the prevalence in our cohort increased to 75% after correction of hypocortisolaemia. Thus assessment of ADH function both before and after glucocorticoid replacement appears to increase the sensitivity for diagnosis of DI in the setting of pituitary metastasis. Overall life expectancy in patients with sellar metastases is 6–22 months.

A compilation of 9 previously reported cases of central DI unmasked by corticosteroids from 2007 to 2017 along with the present 3 cases were performed (Table 1). There was equal gender prevalence with a mean age of 61 (range 56-80 years old). More than 75% of the cases described...
here had previously been diagnosed with advanced malignancies of varying primary sites. The remaining 25% presented with varying symptoms of hypopituitarism as the harbinger to the discovery of the primary neoplasm. Amongst the literature review and cases presented, primary malignancies with pituitary metastasis included lung adenocarcinoma (33%), breast carcinoma (25%), nasopharyngeal carcinoma (16%), renal cell carcinoma (8%), hepatocellular carcinoma (8%) and gastric adenocarcinoma (8%).

Previous studies have reported a high prevalence breast carcinoma and lung carcinoma, however the Asian population shows a significant predilection for pituitary metastasis of nasopharyngeal carcinoma. The prevalence of NPC combined with the progression of disease with direct infiltration of the pituitary gland accounts for the presentation. However, there are no comparable studies looking into the frequency of nasopharyngeal carcinoma with pituitary metastasis.

The common presenting features in the present case series included significant polyuria, polydipsia with some patients presenting with an acute confusional state with the inability to compensate after the initiation of corticosteroids for pan-hypopituitarism. Symptoms of central diabetes insipidus was masked by the relative ADH deficiency. Most cases of pituitary metastasis presenting with central diabetes insipidus as the primary clinical presentation have led to an early diagnosis of pituitary metastasis. The perplexity arises when symptoms are masked and when patients present with symptoms of hyponatremia. Often, these preliminary signs to the presence of pituitary metastasis are often overlooked as these pathognomic symptoms are rare.

Advancement in imaging modalities with interest in neuroimaging has led to precision diagnosis pituitary metastasis. Amongst the literature review and cases presented, initial imaging by CT scan required more comprehensive images requiring MRI. The clinical outcome limited by late presentation in combination with advanced systemic disease should not be a limitation for precision imaging. Oncology offers targeted stereotactic radiosurgery as an effective palliative approach for most patients with pituitary metastasis.[18]

Conclusion

Central diabetes insipidus unmasked by corticosteroids is a less recognized, potentially lethal but fully reversible complication of pituitary metastasis. Symptoms or signs of central diabetes insipidus should be sought in all patient with advanced malignancies presenting with polyuria and hypernatremia. Prompt restoration of pituitary hormones is warranted in affected patients to allow timely restoration of hormonal balance and preventing endocrine emergencies.
Table 1. Brief summary of cases discussed from clinical patients and literature review identifying clinical presentations, investigations, diagnosis and clinical outcome.

| No | age | Sex | Diagnosis                                                                 | Serum osmo mmol/L | Urine osmo mmol/L | Na mmol/L | Clinical outcome                                      | Imaging                                                                 | Notes                                                                 |
|----|-----|-----|----------------------------------------------------------------------------|--------------------|--------------------|-----------|-------------------------------------------------------|-------------------------------------------------------------------------|-----------------------------------------------------------------------|
| CR1| 67  | F   | Diagnosis: lung adenocarcinoma with extensive metastases to liver and bone  | 309                | 115                | 156       | Opted for palliative care succumbing 3 months after admission | MRI showed a lobulated lesion in the right side of the posterior pituitary measuring 0.7 x 1.0 x 0.4cm and a well-defined lesion measuring 0.7 x 0.8 x 0.6cm at the superior aspect of the pituitary infundibulum. |                                                                          |
| CR2| 56  | F   | Diagnosis: nasopharyngeal carcinoma stage Iv with pituitary metastasis      |                    |                    |           | Patient opted for palliative care and succumbed within 1 month. | CT brain revealed increasing size of primary tumour with intracranial extension involving cavernous sinus, pituitary fossa and left temporal and pontine infiltration. |                                                                          |
| CR3| 55  | M   | Diagnosis: nasopharyngeal carcinoma stage Iv with pituitary metastasis      | 294                | 161                | 148       | For palliative chemotherapy with paclitaxel/Carboplatin | CECT Residual tumour at left inferior orbital fissure, leptopectic canal with local infiltration into the cavernous sinus, pituitary sella and right sphenoid sinus |                                                                          |
Presented 3 weeks after initiation of corticosteroids with inability to compensate orally

Polyuric > 150cc urine output per hour

1 71 F Diagnosis: Primary lung malignancy with cerebral and pituitary metastasis diagnosed partial CDI initially obscured by concomitant central hypocortisolism and possible bronchogenic carcinoma-associated SIADH, only becoming overt after steroid replacement.

Clinical Presentation: lethargy, poor oral intake, functional decline and progressive confusion on a background of visual blurring for a number of months.

Low t4 8 pmol/L 8–21
TSH 1.21mIU/L 0.34–5/60
Am Cortisol 147 nmol/L 240–618
LH < 11U/L 11–59
FSH 2 IU/L 17–11

Urine output > 2.7L /day

No biopsy due to advanced malignancy – palliative care
with hypopit replacement transferred to an inpatient hospice on nasal DDAVP and oral dexamethasone

MRI - well defined suprasellar mass measuring 26 mm x 26 mm x 19 mm, and multiple intracerebral hypodensities with perilesional oedema consistent with metastases

Indication for steroid - 1. For allergies cover for CT scan
2. dexa for perilesional oedema

January 2012, the patient died of hypovolemic shock.

MRI of the brain revealed a tumor measuring 13 mm x 13 mm in the sella turcica, which had spread across the suprasellar region

Postmortem examination of the pituitary tumor revealed tumoral hepatocytes in a thick trabecular pattern, the typical appearance of well differentiated HCC

2 80 F hepatocellular carcinoma with pituitary metastasis

GH 0.360 ng/mL 0.010–3.607 ng/mL
PRL 6.83 ng/mL <12.3 ng/mL
TSH 1.288 0.350–4.940 μIU/mL
FT3 1.78 pg/mL 1.71–3.71 pg/mL
FT4 <0.40 ng/dL 0.70–1.48 ng/dL
ACTH 1.4 pg/mL 7.2–63.3 pg/mL
Cortisol 3.1 μg/dL 4.0–19.3 μg/dL
Aldosterone 10 pg/mL 36–240 pg/mL

UA- UA- UA-

March 2015
| Plasma renin activity | 0.5 ng/mL | 0.2–3.9 ng/mL |
|----------------------|-----------|--------------|
| ADH                  | 0.27 pg/mL | 0.3–4.2 pg/mL |
| LH                   | <0.10 mIU/mL | 7.5–56.2 mIU/mL |
| FSH                  | 0.27 mIU/mL | 9.2–124.7 mIU/mL |

Clinical presentation: sudden-onset anorexia accompanied by hypotension and bradycardia. Ix revealed panhypopit. Diabetes insipidus (DI) developed five days into the replacement therapy.

Clinical presentation: with vomiting, low blood pressure and hypoglycaemia. Ix revealed panhypopit. Diabetes insipidus (DI) developed five days into the replacement therapy.

Serum cortisol 12.6 (100-250 ng/mL)
Free T4 6.8 pg/mL (8-18 pg/mL)
TSH 0.005mU/mL (0.5-4 IU/mL),
Testosterone 0.025 (2.5-10 ng/mL),
FSH 0.5 IU/mL (1-8.4 IU/mL),
LH 0.1IU/l (10.5 IU/l),
Low urine osmolarity.

Clinical presentation: 1-day history of confusion, insomnia and reduced appetite. No focal neurological deficit, visual field defect or ophthalmoplegia. Laboratory testing showed hypopituitarism started replacement.

Free thyroxine 4.8 pmol/l
TSH 0.99 miu/l
LH <0.5
FSH <0.5
Prolactin 95 miu/l

Bronchoscopy and biopsy demonstrated a pulmonary adenocarcinoma. Hence we concluded to a lung cancer with multiple pituitary and adrenal gland metastases.

MRI demonstrated an inhomogeneous pituitary hypertrophy, with convexity of the sellar diaphragm, a nodular thickening of the pituitary stalk, and a loss of high intensity signal from the posterior pituitary.

(CT) brain scan was performed, revealing an enhancing (1.5 × 1.7 cm) suprasellar mass with also oedema of the overlying optic tract.

Complicated by cranial DI and SIADH –
Five days after discharge, the patient re-presented to hospital with cranial DI

Primary lung cancer with metastasis to the pituitary was made, complicated by cranial DI

Jan 2016

MRI showed a single 1.2 cm isointense pituitary lesion with loss of the usual posterior lobe hyperintensity on T1-weighted sequence.

Contrast imaging revealed a heterogeneously enhancing pituitary tumor suggestive of intrasellar hemorrhage with a thickened stalk of 3 mm

May 2015

CT head showed 2.6 cm × 1.8 cm × 2.5 cm sellar mass likely with bilateral cavernous sinus extension.

(MRI) was contraindicated due to retained bullet fragments in his left upper chest.

Repeat CT head found that the sellar mass was 3.6 cm × 2.2 cm × 2.5 cm and included a 1.0 cm suprasellar extension that abutted the optic chiasm
| 7 | 66 | F | Clinical Presentation: Hypotensive with sepsis secondary to influenza A/H1N1-pneumonia requiring high inotropic support. Initiated on corticosteroids in ICU. Developed central DI 24 hours after steroid initiation. | 320 | 148 | 153 | No documentation of clinical outcome. Pituitary MRI showed signal increase suggesting hemorrhage in sagittal T1-weighted and coronal T2-weighted MRI, but coronal T1-weighted MRI with contrast showed inhomogeneous enhancement of pituitary with metastasis. |
|---|---|---|---|---|---|---|---|
| 8 * | 56 | F | Clinical Presentation: one month of weight loss and decreased appetite. She had no headaches, increased thirst or visual symptoms. | 312 | 133 | 144 | She was commenced on chemotherapy and brain radiation therapy. Unfortunately, four weeks later, she passed away from cecal perforation and peritonitis. CT of the body and brain showed a mass in the cecum, suprasellar, and extensive intramuscular, bone, lung, lymph nodes and cerebellar deposits. MRI of the pituitary fossa showed a 1.3 x 0.9 cm suprasellar mass inseparable from the pituitary stalk. The pituitary gland was normal. |
| 9 | 64 | M | Clinical presentation: loss of consciousness and gait disturbance. His serum sodium level was 117mEq/L. MRI revealed pituitary metastasis. | UA- | UA- | UA- | Patient underwent subtotal resection of the tumor via a transphenoidal approach. Magnetic resonance imaging revealed a suprasellar tumor that |
with anterior hormone deficiencies. Patient was initiated on steroids and polyuria ensued.

Diagnosis: Pituitary metastasis in a patient with male breast cancer that resulted in pituitary dysfunction.

HPE revealed metastasis from estrogen receptor-positive breast cancer. The patient underwent conventional post-operative radiotherapy combined with hormone replacement therapy and has remained free of symptoms for 16 months.

showed inhomogeneous enhancement and was attached to the optic chiasm.

July 2014
1. Branch CL, Jr, Laws ER, Jr. Metastatic tumors of the sella turcica masquerading as primary pituitary tumors. J Clin Endocrinol Metab. 1987;65:469–474. [PubMed]

2. He W, Chen F, Dalm B et al (2015) Metastatic involvement of the pituitary gland: a systematic review with pooled individual patient data analysis. Pituitary 18:159–168. https://doi.org/10.1007/s11101-014-0552-2

3. Ref Komninos J, Vlassopoulou V, Protopapa D, Korfias S, Kontogeorgos G, Sakas DE, Thalassinos NC. Tumors metastatic to the pituitary gland: case report and literature review. J Clin Endocrinol Metab. 2004;89:574–80. doi: 10.1210/jc.2003-036395. [PubMed] [Cross Ref]

4. Delarue J, Chomette G, Pinaudeau Y, Brocheriou C, Auriol M (1964) Pituitary metastases. Frequency. Arch Pathol. (Paris) 12:179–182

5. Twelve cases of pituitary metastasis: A case series and review of the literature. Mendel Castle-Kirsbaum Tony Goldsclager Benjamin Ho Yi Yuen Wang James King3 Springer Science+Business Media, LLC, part of Springer Nature 2018

6. Neurosarcoediosis-associated central diabetes insipidus masked by adrenal insufficiency Lemuol Non1, Daniel Britol, Catherine Anastasopoulos2 BMJ Case Rep. 2015; 2015: bcr201426390. Published online 2015 Jan 22. doi: 10.1136/bcr-2014-206390. PMID: PMC4307084 PMID: 25612752

7. Ishikawa SE, Fukagawa A, Higashiyama M et al (2001) Close association of urinary excretion of aquaporin-2 with appropriate and inappropriate arginine vasopressin-dependent antidiuresis in hyponatremia in elderly subjects. J Clin Endocrinol Metab 86:1665–1671. https://doi.org/10.1210/jcem.86.4.7426

8. HX Chio, TPL Quek, MKS Leow . Central diabetes insipidus unmasked by corticosteroid therapy for cerebral metastases: beware the case with pituitary involvement and hypopituitarism. JOURNAL OF THE ROYAL COLLEGE OF PHYSICIANS OF EDINBURGH VOLUME 47 ISSUE 3 SEPTEMBER 2017 247 – 9 doi: 10.4997/JRCPE.2017.307 Endocrine Society’s 97th Annual Meeting and Expo, March 5–8, 2016 - San Diego

9. Pituitary metastasis of hepatocellular carcinoma presenting with panhypopituitarism: a case report. Tomoko Tanaka, Katsushi Hiramatsu, Takuto Nosaka, Yasushi Saito, Tatsushi Naito, Kazuto Takahashi, Kazuya Oyaji, Hitetaka Matsuda, Masahiro Ohtani, Tomoyuki Nemoto, Hiroyuki Suto, Tatsuya Yamamoto, Hirohiko Ka,

Yasunari Nakamoto BMC Cancer. 2015; 15: 863 Published online 2015 Nov 6. doi: 10.1186/s12885-015-1745-y

10. An acute adrenal insufficiency revealing pituitary metastases of lung cancer in an elderly patient Hela Marmouch, 1, 1 & Sondes Arfa, 1 Saoussen Cheikh Mohamed, 2 Tems Slim, 1 and Ines Khochtali Pan Afr Med J. 2016; 33: 16. Published online 2016 Feb 8. doi: 10.11604/pamj.2016.23.34.8905

11. Competing interests in a lung cancer with metastasis to the pituitary gland: syndrome of inappropriate ADH secretion versus diabetes insipidus. Oxf Med Case Reports. 2016 Jan; 2016(6): 125–129. Published online 2016 Jun 1. doi: 10.1093/omcr/omw044 PMID: PMC4887828 PMID: 27274855 Gaurav Singh Gulsin, 1, * Madeleine Louisa Bryson Jacobs, 2 Shailesh Gohil, 3 Adam Thomas, 4 and Miles Levy, 3

12. Symptomatic Metastasis to the Pituitary Gland: A Report of Three Cases and Review of the Literature. J Neurol Disord. 23: 236. doi: 10.4172/2329-6733.1000236 Yung C, Timothy SKC, Peter KHP, et al. (2015)

13. Isolated pituitary metastasis from renal cell carcinoma in a horseshoe kidney Kay J Win, Nissa Blucher, William Tester, Sergey Ginzburg, Lauren Pomo Journal of Solid tumor 2016 3: 236. doi: 10.4172/2329-6733.1000236

14. Acute-Onset Panhypopituitarism Nearly Missed by Initial Cosyntropin Testing Claudine A. Blum, 1, 1 DanieL Schneeburger, 1 Matthias Lang, 1 Janko Rakic, 1, 2 Marc Philippe Michot, 1 and Beat Müller 1, 1 Medical University Clinic, Kantonsspital Aarau, Aarau, Switzerland 2 Department of Pneumology, Medical Clinic, Kantonsspital Baden, Baden, Switzerland Published 3 October 2017 Case Reports in Critical Care Volume 2017, Article ID 7931438, 4 pages https://doi.org/10.1155/2017/7931438

15. PITUITARY METASTASIS SECONDARY TO OCCULT BREAST MALIGNANCY: A CASE REPORT Kalpana Vijakumar, MBBS, Su Ping Brenda Lim, MBBS, MRCP, Wai Han Hol, MBBS, MRCP Tan Tock Seng Hospital ABSTRACTS – Pituitary Disorders/Neuroendocrinology Jan 2016

16. A case of pituitary metastasis in a patient with male breast cancer developing anterior lobe dysfunction successfully treated by using hormone replacement therapy Fukunaga A1, Yazaki T, Shimizu K, Ochiai M. Department of Neurosurgery, Kyoai Tachikawa Hospital. No Shinkei Geka. 2014 Jul;42(7):629-33. PMID: 25006103

17. Benjamin. L.: Ein Krebs Fall. Virchows Arch. Path. Anat. 12 (857) 566-569

18. Stereotactic radiosurgery for pituitary metastases. Surg Neurol. 2009 Sep;72(3):248-55; discussion 255-6. doi: 10.1016/j.surneu.2008.06.003. Epub 2008 Sep 11 Kano H1, Niranjan A, Kondziolka D, Flickinger JC, Lunsford LD.
Euglycemic diabetic ketoacidosis reported in pregnant women.

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Abstract

Pregnancy is a physiological state in women which creates significant alterations in energy metabolism to provide energy supply to the foetus. The maternal fasting glucose level would be less than that of a non-pregnant state and in contrast plasma ketones and free fatty acids levels are elevated, resulting in a state of accelerated starvation. These metabolic alterations place a pregnant woman at a higher risk of developing euglycemic ketoacidosis. We report a rare case of euglycemic ketoacidosis causing severe increased anion gap metabolic acidosis in a pregnant woman.

Keywords: Euglycemic ketoacidosis, pregnancy

Introduction

Diabetic Ketoacidosis (DKA) is defined as presence of moderate serum or urine ketone bodies, serum glucose above 250mg/dL, arterial pH less than 7.3 and serum bicarbonate below 18mEq/L [1] and it causes serious metabolic complication with high mortality rate. DKA can occur in pregnancy and lead to compromise in both the mother and fetus [2]. The hormonal changes in pregnancy create a state of insulin resistance allowing free flow of glucose to the foetus. Thus, prolonged starvation will also place her at high risk of starvation ketosis.

Here we present a pregnant patient with type 1 diabetes presenting with euglycemic diabetic ketoacidosis.

Case report

A 29year old primigravida mother was presented at her 29weeks of gestation to surgical ward with fever, left sided lower limb swelling and persistent vomiting for 2days duration. Initial obstetric evaluation was focused on excluding possibility of an obstetric emergency. She had type 1 diabetes mellitus which required bolus insulin regime three times daily and basal bolus regime at night. A clinical diagnosis of cellulitis was made and was treated with intravenous clindamycin. Upon examination, she was found to be unwell and persistently tachypneic; however, her haemodynamic status was stable. Her capillary blood sugar was 135 mg/dL and ABG showed pH of 7.1, pCO2 of 7.2mmHg, pO2 of 110.5mmHg, HCO3- of 10.5mmol/L and lactate of 0.9mmol/L. Urine ketone bodies were 3+. Serum electrolytes were Na+ – 138 mmol/L, K+ – 3.3 mmol/L and Cl− – 100 mmol/L. She had persistently low lactate level with normal liver biochemistry and renal profile which made sepsis a remote possibility to cause severe increased anion gap metabolic acidosis. However, further metabolic workup related to acidosis showed euglycemic ketoacidosis and she was managed according to usual diabetes ketoacidosis protocol. Fluid resuscitation with 1L of 0.9% saline was given over one hour and later converted to 0.45% saline and 5% dextrose infusion at a rate of 250mL/hour for next 6hours and later converted to 100mL/hour infusion. Intravenous soluble infusion (0.1U/hr) was started at 6U/hr with intravenous potassium replacement. We continued insulin infusion for nearly 72hours until patient improved with no vomiting and improved appetite with negative urine ketone bodies. Later insulin was converted to subcutaneous soluble insulin.

Discussion

Patients with type 1 diabetes can present with features of diabetic ketoacidosis without hyperglycemia which is defined as euglycemic diabetic ketoacidosis. Reduced hepatic production of glucose during fasting or increased urinary excretion of glucose by counter regulatory hormones causes euglycemic diabetic ketoacidosis [3].
Infections, poor adherence to treatment, persistent vomiting, corticosteroid therapy and beta sympathomimetic drugs are common precipitating factors for DKA in pregnancy [2]. The patient with a trigger for diabetic ketoacidosis, taking regular insulin levels despite not taking meals, liver in a state on depleting glycogen causes less glucose production. Moreover, lipolysis with fatty acid production causes ketone production and lead to euglycemic diabetic ketoacidosis [3]. Common causes of euglycemic DKA are pregnancy, low calorie intake, fasting or starvation, pancreatitis, SGLT2 inhibitors and prolonged vomiting [4]. In our case, poor intake of meals due to infection, despite taking regular insulin causes lipolysis and subsequently more productions of ketone bodies lead to euglycemic diabetic ketoacidosis.

Diagnosis of euglycemic diabetic ketoacidosis is made after exclusion of other causes of high anion gap metabolic acidosis. In our case, patient’s lactate level is normal. Patient did not have evidence of renal failure or any ingestions of alcohol or salicylates. Early clinical suspicion of euglycemic diabetic ketoacidosis and prompt treatment lead to less mortality. Treatment is same as management of diabetic ketoacidosis. Treatment compromise of adequate fluid resuscitation, continuing insulin with dextrose infusion until clinical improvement, normalizing bicarbonate and anion gap. [5]

References
1. Nyenwe EA, Kitabchi AE. The evolution of diabetic ketoacidosis: an update of its etiology, pathogenesis and management. Metabolism 2016; 65; 507–21.
2. Kamalakannan D, Baskar V, Barton DM, et al. Diabetic ketoacidosis in pregnancy Postgraduate Medical Journal 2003; 79:454–7.
3. Kitabchi AE, Umpierrez GE, Miles JM, Fisher JN. Hyperglycemic crises in adult patients with diabetes. Diabetes Care 2009;32: 1335–43.
4. Rawla P, Vellipuram A, Bandaru S, Pradeep Raj J. Euglycemic diabetic ketoacidosis: a diagnostic and therapeutic dilemma. Endocrinology, Diabetes & Metabolism Case Reports 2017;15; 201-7.
5. Gelaye A, Haidar A, Kassab C, Kazmi S, Sinha P. Severe ketoacidosis associated with canagliflozin (Invokana): a safety concern. Case Reports in Critical Care 2016; 2016:1656182.

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Informed consent
Informed written consent was obtained from the patient for her anonymized information to be published in this article retrospectively.
Look beyond What You See – Lessons Learnt in Endocrinology

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A 36-year-old male presented with blurred vision of his left eye. The appearance of his fundus is shown in figure (1a). Four years later, he presented with acute right-sided loin pain and during this episode, he was also found to have incidental hypertension of 140/100 with tachycardia. The contrast enhanced computerized tomography scan done during this admission, shown in figure (1b), revealed a contrast enhancing soft tissue mass of 8.1x4.7x4.1cm and 5x3.5x4.4cm in the right and left suprarenal regions, respectively. There were multiple hypertrophied vascular channels around the lesions. In addition to these 2 lesions, there was a heterogeneous enhancing soft tissue mass of 15mm in diameter in the right renal cortex (fig 1b). The magnetic resonance imaging of the brain showed a contrast enhancing 0.4cm lesion in the right cerebellum as seen in (fig 1c).

What is the diagnosis?

Von Hippel Lindau disease

Von Hippel Lindau (VHL) is a rare autosomal dominant syndrome. Retinal and cerebellar haemangioblastomas, clear cell renal carcinomas, pheochromocytomas, pancreatic endocrine tumours and endolymphatic sac tumours are commonly seen among VHL patients. Retinal angiomas are the most common presenting feature of VHL and are frequently multiple and bilateral, usually present in mid-twenties. Dilated and tortuous vessels leading to and from the tumour are characteristic. Continuous growth of the tumour can lead to retinal hard exudates, retinal oedema and subsequently retinal detachments (2). CNS haemangiomas are seen in 40% of cases and usually arise in early thirties (2). These are usually found in the spinal column, cerebellum and the brain stem. Pheochromocytoma is found in 10-20% of VHL patients. Mean age of presentation is 30 years. They can be multiple and bilateral. (4). Renal cell carcinomas are the commonly seen malignant neoplasms. The mean age of presentation is 39 years. Occasionally, advance tumours can present with flank pain as was seen in the indexed patient (3).

Deciding on the best management option for the bilateral pheochromocytomas was a challenging decision in this patient. Bilateral adrenalectomy invariably pushes the patient into lifelong steroid dependence and associated poor quality of life, with a lifelong risk of life threatening adrenal crisis. Cortical sparing adrenalectomy might be helpful in mitigating this problem. However, the probability of recurrence outweighs the benefits in these kinds of hereditary pheochromocytomas. This is an inherited disorder with multiple problems at different ages of life and this case underscores the importance of regular surveillance in VHL disease.
1. Dollfus H, Massin P, Taupin P, Nemeth C, Amara S, Giraud S et al. Ocular manifestations in von Hippel-Lindau disease: a clinical and molecular study. Investigative Ophthalmology and Visual Science 2002; 43: 3067–3074.
2. Maher ER, Yates JR, Harries R, Benjamin C, Harris R, Moore AT et al. Clinical Features and Natural History of von Hippel-Lindau Disease. Quarterly Journal of Medicine 1990; 77: 1151–1163.
3. Walther MM, Reiter R, Keiser HR, Choyke PL, Venzon D, Hurley K et al. Clinical and genetic characterization of pheochromocytoma in von Hippel-Lindau families: comparison with sporadic pheochromocytoma gives insight into natural history of pheochromocytoma. Journal of Urology 1999; 162: 659–64.
Clinical Practice Guideline

Non-alcoholic fatty liver disease - 2019

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Introduction

This clinical practice guideline on the evaluation and treatment of non-alcoholic fatty liver disease (NAFLD) among adults is a product of a joint effort by The Sri Lanka College of Endocrinologists (SLCE) and Sri Lanka Society of Gastroenterology (SLSG). This guideline is based on extensive, up-to-date evidence search made by the guideline team and selected to match the clinical context and available resources. The ultimate purpose of the guideline is to increase awareness and to improve patient care of non-alcoholic fatty liver disease (NAFLD), and to assist caring physicians in the decision-making process by providing evidence-based guidance considering the health care burden posed by the management of the disease.
Definitions

NAFLD is defined as the presence of hepatic steatosis (HS) (fatty liver, >5% fat in the liver), in the absence of any secondary cause for HS. Exclusion of ‘unsafe’ alcohol consumption above Asian standards (>14 units per week for males and >7 units per week for females) is essential for the diagnosis. In appropriate situations, other causes of HS such as chronic viral hepatitis, autoimmune and hereditary liver disease, and contribution of steatogenic drugs should be also excluded.(1)

HS is most commonly established by liver ultrasonography. Less commonly, other imaging modalities such as CT or MRI scanning will be required to establish HS. Liver biopsy and histology is rarely required for the diagnosis. NAFLD is a spectrum of disorders ranging from simple steatosis [non-alcoholic fatty liver (NALD)], non-alcoholic steatohepatitis (NASH) and NASH-related cirrhosis.

Table 1. Definitions of NAFLD

| NAFLD (Non-alcoholic fatty liver disease) | There should be evidence of hepatic steatosis (HS), either by imaging or histology and absence of secondary causes of hepatic fat accumulation such as significant alcohol consumption, long term use of a steatogenic medication, hereditary and autoimmune liver disorders.(2) |
|-----------------------------------------|------------------------------------------------------------------------------------------------------------------|
| NAFL (Non-alcoholic fatty liver)        | The presence of HS without evidence of hepatocellular injury (hepatocyte ballooning)                                  |
| NASH (non-alcoholic steatohepatitis)    | The presence of HS and inflammation with hepatocyte injury (ballooning), with fibrosis (F1-4) or without (F0) fibrosis |
| Lean NAFLD                              | NAFLD that develops in patients with a body mass index (BMI) <23 kg/m2(3,4)                                         |
| NASH cirrhosis                          | The presence of cirrhosis with current or previous histological evidence of steatosis or steatohepatitis            |

Note: Fibrosis Severity Scale: F0 - No fibrosis, F1 - Mild fibrosis, F2 - Moderate fibrosis, F3 - Severe fibrosis, F4 - Cirrhosis

Incidence and Prevalence of NAFLD

The incidence and the prevalence of the NAFLD have risen exponentially in the recent past (Figure 1). In Sri Lanka, the prevalence of ultrasonically detected NAFLD among urban adults is 32.6% (6) and among rural adults is 18% (7). The annual incidence of NAFLD among urban adults is 6.6% (8)
Pathogenesis and pathophysiology

Fatty liver is considered to be the hepatic component of metabolic syndrome. Although the pathogenesis of NAFLD/ NASH is not yet fully understood, several theories are currently available to describe its development. Steatosis represents the ‘first hit’, which then sensitizes the liver to injury mediated by ‘second hits’, such as inflammatory cytokines, adipokines, oxidative stress, angiotensinogen, norepinephrine, osteopontin and mitochondrial dysfunction, leading to steatohepatitis and fibrosis. Furthermore, accumulation of FFA alone has been suggested to be sufficient to induce liver damage, without recourse for a second hit. Oxidative stress reduces the ability of mature hepatocytes to proliferate leading to increased hepatocyte death (9). Additionally, several agents including leptin, insulin, glucagon, deacylated ghrelin, selenoprotein P levels are increased while levels of adiponectin, GLP 1, acylated ghrelin are decreased in NAFLD contributing to the pathophysiology of the disease as described in (figure 2) (10).

There seems to be a genetic predisposition (11) to the development of NALFD. Some of the established association of genetic variants for development and progression of fibrosis in NAFLD include PNPLA3 (patatin-like phospholipase domain-containing protein 3), TM6SF2 (trans-membrane-6 super-family-2) and MBOAT7 (membrane bound O-acetyltransferase domain containing 7). Out of these variants PNPLA3 (12) has been proven to be associated with NAFLD in Sri Lanka.

Recently there is accumulating data that the gut microbiome may play a key role in the development and progression of NALFD via lipopolysaccharides, inflammatory cytokines and bile acid derivatives (12).
Figure 2: Pathophysiology of the development of NAFLD

Figure 2: Pathophysiology of the development of NAFLD
NAFLD is strongly associated with metabolic syndrome as well as its individual components.

**Obesity**

Obesity carries a 3.5 fold increased risk of developing NAFLD (13). Prevalence of NAFLD among obese individuals is 70%. Further, obese NAFLD, when compared to non-obese NAFLD has a higher transaminases, higher degree of hepatic steatosis and increased risk of liver fibrosis, hypertension, diabetes mellitus, and metabolic syndrome (14). However, some individuals with NALFD will have normal BMI (<23kg/m2). They are referred to as lean NALFD and accounts for up to 12% of NALFD in Sri Lanka (15).

**Type 2 diabetes mellitus**

The overall prevalence of NAFLD among T2DM is 70 % (16). 2-5 times increased risk of incidental T2DM is demonstrated in NAFLD and it’s incidence reduces with resolution of fatty liver (17). However, T2DM and NAFLD can develop almost simultaneously in a patient due to the common risk factors for both conditions, confounding the prevalence of NAFLD in patients with T2 DM or vice versa.

**Dyslipidemia**

Nearly half of the patients with dyslipidemia were found to have NAFLD, with higher prevalence in those with high triglycerides and low HDL levels. (High TG: HDL ratio and/ or high Total cholesterol/HDL ratio) (18).

**Malignancies**

NAFLD increases the risk of hepatocellular carcinoma by 10 times. The presence of NAFLD doubles the risk of colon cancers in men and breast cancers in women(19).

**Other associations**

NAFLD increase the risk IHD by 1.5 times and chronic kidney disease by 2 times (20). Polycystic ovarian syndrome and hypothyroidism are other known associations of NAFLD.

**Death**

The leading cause of mortality among patients with NAFLD is cardiovascular disease. This is followed by cancer-related deaths and liver-related deaths. These deaths are strongly associated with the presence of significant or advanced liver fibrosis (>F2 – bridging fibrosis or cirrhosis) rather than the presence of NAFL/NASH alone (21).

**Natural history and outcomes**

The progression of NAFLD ranges from simple steatosis to hepatic inflammation, subsequent fibrosis with development of cirrhosis, and hepatocellular carcinoma (HCC). The time for progression from one stage to another varies according to the underlying disease stage. Natural history of the NAFLD is summarized in Figure 3.

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**Figure 3: Natural history and outcomes of NAFLD and risk factors for disease progression**
Cardiovascular disease is the leading cause of mortality in NAFLD(21). This is followed by cancer-related deaths due to extra-hepatic malignancies such as colorectal cancer in males and breast cancer in females as the second common cause of death in NAFLD. Liver-related deaths are only the third most common cause of death in NAFLD(22). This highlights the importance of screening and treating associated cardiovascular risk factors and screening for appropriate malignancies among patients with NAFLD.

Patients with NASH have six-time higher mortality compared to those with isolated NAFL(23, 24). Similarly, patients with NASH and significant liver fibrosis have worse outcomes compared to those without significant liver fibrosis. Presence of significant liver fibrosis will increase all cause, cardiovascular and liver related mortality in NAFLD(25-27).

Screening

Screening for NAFLD can be recommended in ‘high risk’ patients such as obese and those with type 2 diabetes, dyslipidemia and metabolic syndrome, due to their strong association. Persons with persistently abnormal liver enzymes also should be screened for NAFLD (28). Some lean individuals are also prone to get NAFLD (lean NAFLD). Therefore, vigilant screening of lean individuals with other features of metabolic syndrome should be practiced(29). Routine population screening or family screening is not recommended due to lack of evidence of benefit(30).

Ultrasound abdomen is recommended as an initial investigation for screening of NAFLD as it is non-invasive, easy to perform, cheap and readily available (28,31).

Evaluation

Evaluation of NAFLD includes diagnosis and staging of the liver disease and assessment of risk factors and associated diseases.

The diagnosis of NAFLD requires the evidence of HS on imaging or histology in the absence of other causes of steatosis or liver disease (30). Initial evaluation should include detailed medical, medication and family history to evaluate for risk factors, alternative etiology for liver disease and complications. Details of daily activity, physical exercise, dietary habits, alcohol and smoking habits, medical co-morbidities and current medications should be established.

General physical examination including weight, BMI to assess general obesity (23-24.9Kg/m² – over-weight, >25kg/m² – obesity), waist circumference to assess central obesity (>90cm in males, >80cm in females)(32), blood pressure, features of insulin resistance, findings suggestive of other etiologies of liver disease should be documented (Table 2) (28,30,33). Fasting blood sugar or HbA1c and fasting lipid profile should be performed routinely to identify those with associated diabetes and dyslipidemia.

| Secondary causes of hepatic steatosis | Alternative causes of liver disease |
|---------------------------------------|-----------------------------------|
| ‘Unsafe’ alcohol consumption           | Alcohol                            |
| Steatogenic medications -              | Hepatitis B/C infections           |
| (corticosteroids, amiodarone, methotrexate, tamoxifen, valproate, antiretroviral) | Hemochromatosis                     |
| Chronic hepatitis C infection          | Wilson disease                     |
| Parenteral nutrition                   | Autoimmune liver diseases          |
| Severe malnutrition                    | Drugs                              |
| Pregnancy associated liver disease     | Alternative medicines              |
| Abetalipoproteinemia                   | Alpha-1 antitrypsin deficiency     |

Table 2. Common causes for hepatic steatosis and liver disease
Risk stratification to determine the stage of NAFLD will help to predict the progression of the disease, prognosis and identify the treatment candidates. This should be initially undertaken non-invasively with locally available tests (Table 3). High risk patients may be subjected to further evaluation including liver biopsy (28, 30, and 33).

### Table 3. Non-Invasive Investigations for the evaluation of NAFLD

| INVESTIGATION                          | STRENGTHS                                                                 | LIMITATIONS                                                                 |
|----------------------------------------|---------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Liver profile                          | • Mildly raised transaminases (ALT > AST) and for Gamma glutamyl transferase | • ALT values do not correlate with histological findings and severity       |
|                                        |                                                                          | • ALT can be normal in >50% of individuals with NASH and 80% of individuals with NAFLD |
| Ultrasound abdomen                     | • Widely available and cheaper                                           | • Cannot distinguish between the stages of NAFLD.                          |
|                                        | • Additional assessment of hepato-biliary system                          | • Suboptimal sensitivity and specificity for mild steatosis (<30%)          |
|                                        | • Sensitivity: 53-100%                                                    | • Not reliable in obese individuals                                        |
|                                        | Specificity: 77-98%                                                      |                                                                           |
| MRI                                    | Liver MRI scan                                                           | • High cost                                                              |
|                                        | • Gold standard among currently available tools.                          | • Needs expertise                                                        |
|                                        | MR Spectroscopy                                                          | • Not widely available                                                    |
|                                        | • Quantitative estimation of liver steatosis.                             |                                                                           |
|                                        | • Useful in research setting but not in clinical practice.               |                                                                           |
|                                        | • Sensitivity: 77% to 100%                                               |                                                                           |
|                                        | Specificity: 87% to 91%                                                  |                                                                           |
| MRI Elastography                       | The most accurate imaging method to identify varying degrees of fat infiltration and fibrosis |                                                                           |
|                                        | • Sensitivity: 75% to 88%                                                |                                                                           |
|                                        | Specificity: 85% to 90%                                                  |                                                                           |
| Fibro scan (VCTE)                      | • Point of care tool                                                     | • Suboptimal sensitivity and specificity for mild fibrosis               |
|                                        | • Can rule in/rule out advanced fibrosis                                 | • Learning curve for technique                                            |
|                                        | • Sensitivity: 95%                                                       | • Unreliable results in the presence of high BMI and thoracic fold thickness|
|                                        | Specificity: 77%                                                         |                                                                           |
| Serum based scores                     | • Calculators are freely available in web                                | • Not validated in Sri Lankan population.                                  |
| (NAFLD Fibrosis score (NFS) and        | The negative predictive values for excluding advanced fibrosis are       |                                                                           |
| Fib-4 index)                           | higher, therefore, can exclude advanced fibrosis                         |                                                                           |

**Note:** FIB-4 comprises age, platelets and AST/ALT; NFS includes age, BMI, DM, platelets, AST/ALT, albumin. Based on the non-invasive assessment patients should be categorized to ‘Low risk of fibrosis or ‘High risk of fibrosis. Low risk of fibrosis – FibroScan (TE)<5 kPa, FIB-4<1.3, NFS<-1.455. High risk of fibrosis – FibroScan (TE)>10 kPa, FIB-4>3.25, NFS>0.676.
Liver biopsy

Liver biopsy is the only method to reliably diagnose NASH and is the only investigation that reliably differentiates the stages of NAFLD. However it is invasive, expensive, requires expertise for interpretation and carries some morbidity and rarely mortality related to the procedure (28, 30, 33, 34).

Liver biopsy may be considered in NAFLD/NASH in the following settings: 1. To exclude competing or coexisting etiologies for liver disease 2. Advanced fibrosis which is suggested by serum based scores and/or imaging 3. To establish the diagnosis of NASH in patients with poor response to therapy

Histology

Clinically useful pathology report should distinguish between simple steatosis and steatohepatitis. NAFLD encompasses: steatosis alone, steatosis with lobular or portal inflammation without ballooning or steatosis with ballooning but without inflammation. The diagnosis of NASH requires the joint presence of steatosis, ballooning and lobular inflammation. A comment on severity based on specific scoring systems such as NAFLD activity score (NAS) is useful. The presence of fibrosis should be described, and if present, a further statement related to location, amount and parenchymal remodeling is warranted (28, 30, 33, and 34).

Management strategies Management of NAFLD should ideally be via a multidisciplinary approach (30). This will increase the rate of diagnosis, improve metabolic measures and hepatic/non-hepatic outcomes and reduce severity, which will be cost effective. This multi-disciplinary team will include Gastroenterologists/Hepatologists, Endocrinologists, Cardiologists, Bariatric Surgeons, Nutritionists, Behavioral experts and specialist nurses. Whenever possible, patients should be managed by such Multidisciplinary team (MDT).

A successful management strategy should be tailor-made to the stage of the disease in the spectrum of NAFLD and may include following key components:

1. Non-Pharmacological treatment
   1.1 Achieving target weight loss
   1.2 Regular physical exercise
   1.3 Healthy dietary modifications
   1.4 Bariatric interventions

2 Pharmacological treatment

3 Management of risk factors and metabolic associations
Algorithm 1: Evaluation of NAFLD
Non-pharmacologic management in the form of healthy lifestyle modifications is the cornerstone in management of NAFLD and prevention of its progression. A comprehensive lifestyle approach to management of NAFLD focuses on three key areas: weight loss, dietary modifications, and exercise which in combination will provide the optimum benefit in all patients with NAFLD. The sustainability and consistency of this lifestyle approach over a long period of time should be ensured through persistent motivation and cognitive behavioral therapy.

1.1 Weight Loss

Weight loss has shown a significant improvement of clinical and histological parameters of NAFLD. 5-7% weight loss will result in resolution of steatosis. 7-10% weight loss will result in resolution of steatohepatitis. More than 10% weight loss will result in regression of fibrosis. Thus, a target weight loss of at least 5-10% is recommended to be achieved via a combination of diet and exercise(28).

Degree of weight reduction in an individual is positively associated with the resolution and improvement of liver histology (Figure 6) (35,36,37,38).

1.2 Dietary modifications

The primary aim of dietary modifications is to achieve the target weight loss through a hypo-caloric diet. However, macronutrient and micronutrient composition in diet have also shown to improve biochemical and histological manifestations of NAFLD independent of weight loss.
Figure 5: Comprehensive lifestyle approach to NAFLD management

Figure 6: Effect of weight loss on histological improvement of NAFLD

Figure 5: Effect of weight loss on histological improvement of NAFLD
1.3 Exercise and Physical Activity

Moderate-intensity exercise for 30 – 60 minutes on 3–5 days per week is recommended (28,41). However, the intensity, duration and type of exercise should be individualized based on patient's baseline cardiovascular fitness, preference and lifestyle. (Algorithm 2)

Exercise helps to reduce NAFLD progression by reduction of mean hepatic triglyceride composition, peripheral adipose tissue volume, free fatty acid concentration and increase of hepatic glucose sensitivity. The level of cardiovascular fitness at baseline is shown to be the most important factor to affect the degree of hepatic steatosis and it is shown to predict the improvement in NAFLD following lifestyle interventions (44). Benefits of exercise in NAFLD depend on the duration, intensity and type of exercise, independent on the degree of weight loss. Vigorous intensity exercise (Annexure 1) is beneficial in reducing the likelihood of developing NASH and its progression and moderate intensity exercise has shown to have some benefits as well (45,46). Aerobic exercise and resistant training have shown similar benefits in reduction of progression of NASH(47). Apart from improvement of NAFLD, exercise leads to reduction in the overall cardiovascular risk.
### Table 4. Non-recommendations for dietary and lifestyle interventions

| Dietary interventions | Mechanism of benefit | Recommendation |
|-----------------------|----------------------|----------------|
| Hypo-caloric diet     | Calorie restriction drives weight loss and the reduction of liver fat, independent of the macronutrient composition of the diet. | 500-1000 kcal energy deficit per week is recommended to induce 0.5 to 1 kg of weight loss. (28) |
| **LOW CARBOHYDRATE (<40%) DIET**<br>• Low carbohydrate diet: calories should be replaced with MUFA, and proteins from fish, meat, nuts and legumes. | **BENEFITS OF A LOW CARBOHYDRATE DIET**<br>• Reduces glycemic load <br>• Reduces insulin resistance <br>• Reduces triglycerides and increases HDL. | • Either a low carbohydrate or a low fat diet is recommended as both types of diets have shown similar benefits. The choice can be based on patient’s preference and feasibility(39) |
| **LOW FAT (<30%) DIET**<br>• Low fat diet: calories should be replaced with low glycemic index food items and protein containing food. | Fructose metabolism has shown to increase lipogenesis, hepatocyte steatosis, and oxygen free radical production (40). | • Sugar sweetened beverages with high fructose corn syrup (eg: soft drinks) should be minimized or avoided |
| Minimize fructose containing food | | |
| Minimize / avoid fast food | Food items with high energy density, large portion size, high carbohydrate content, low fibre, high fructose content, red meat and food high in trans-fat will cause over flow of fat in to liver and hepatic inflammation (41). | • Consumption of fast food should be minimized or avoided |
| **Lifestyle interventions** | | |
| Alcohol consumption | Should be discouraged as any degree of alcohol will result in progression of fibrosis and poor outcomes(42) | |
| Coffee consumption | • Regular black, unsweetened drip coffee 2-4 cups has shown to improve liver enzymes, reduce the risk of NAFLD, liver fibrosis and HCC(43). | • Current evidence is inadequate to provide a strong recommendation but can be used as an adjunct |
| Sleep | • 7-8 hours of adequate sleep will be beneficial | • Current evidence is inadequate to provide a strong recommendation but can be used as an adjunct |
Algorithm 2: Decision making on the exercise regimen in NAFLD

Algorithm 2: Decision making on the exercise regimen in NAFLD. *annexure 2
1.4 Bariatric (Metabolic) intervention

Bariatric intervention (endoscopic or surgical) could be considered in patients aged between 18-65 years with a BMI $\geq 30$ kg/m$^2$ with NAFLD. Although not to be used solely for the treatment of NAFLD, bariatric surgery can be useful in management of obese patients with other co-morbidities or indications.

Weight reduction is associated with significant improvement in liver histology in patients with NAFLD by several postulated mechanisms (Figure 7)(48). Bariatric surgery is more efficacious in achieving weight loss of 10 to 30% in morbidly obese patients when compared to diet, lifestyle modifications and anti-obesity medications (49). Bariatric surgery related weight loss resolved NASH in up to 85% of patients (50, 51,52) and improved liver fibrosis in 34% at 1 year post operatively (50). NAFLD is considered as an important comorbidity when deciding on bariatric surgery in morbidly obese individuals (6, 10,11).

The presence of cirrhosis is a relative contraindication for bariatric surgery, as it might lead to increased mortality (48,53,54).

Pharmacologic management

Liver-directed pharmacological therapies are only considered for patients with NASH with or without advanced fibrosis. Pharmacological treatment lack evidence in phase III trials, hence not approved for NAFLD by any regulatory authorities. Several categories of pharmacological treatments are available with variable degree of evidence (Table 5). Off label use of pharmacologic therapy might be considered in following stages of NAFLD depending on the excess mortality (27,55) safety and tolerability.1. NASH with fibrosis.2. NASH with high necro-inflammatory activity3. NASH with increased risk of progressing to fibrosis (Age>$50$years, Diabetes mellitus, Metabolic syndrome, persistently increased ALT).
| Medication                      | Remarks                                                                 | Adverse effects                                                                                   | Recommendations                                                                                      |
|--------------------------------|-------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|
| **Pharmacologic agents with proven benefits**                                                                                                                                     |
| **Hepato-protective agents**   |                                                                         |                                                                                                  |                                                                                                   |
| Antioxidant Vitamin E          | • Reduction in aminotransferases                                        | • May increase risk of prostate cancer, hemorrhagic stroke and all-cause mortality                | • Recommended for biopsy proven NASH in non-cirrhotic and non-diabetics for maximum duration up to 2 years (800 IU/daily) |
|                               | • Improvement in steatosis, inflammation and ballooning                  |                                                                                                  |                                                                                                   |
|                               | • Resolution of steatohepatitis in a proportion of non-diabetic adults with NASH |                                                                                                  |                                                                                                   |
|                               | • No effect on hepatic fibrosis (56,57)                                 |                                                                                                  |                                                                                                   |
| **Anti-diabetic agents**       |                                                                         |                                                                                                  |                                                                                                   |
| Thiazolidinedione (PPAR agonist) | **Pioglitazone (58)**                                                   | Concerns regarding weight gain, congestive cardiac failure, increased risk of bladder cancer and fracture | • Recommended for biopsy proven NASH after discussing risks and benefits                           |
|                               | • Improvement in liver histology including the fibrosis score and arresting disease progression |                                                                                                  | • Not recommended for NAFL alone                                                                   |
|                               | • Improvement in insulin resistance and transaminase levels             |                                                                                                  |                                                                                                   |
|                               | • Shown benefits in both patients with diabetes and without diabetes     |                                                                                                  |                                                                                                   |
| **Pharmacological agents with NO proven benefits**                                                                                                                               |
| Metformin                      | • Improvement in insulin resistance but no improvement in liver histology (59)(60) | Gastro-intestinal effects                                                                        | NOT recommended for NAFLD alone                                                                  |
|                               | • Reduces HCC risk (61)                                                 |                                                                                                  |                                                                                                   |
| Glucagon-like peptide-1 analogue | **Liraglutide (62): histological resolution of NASH**                   | Gastro-intestinal effects                                                                        | Limited evidence to recommend at present                                                         |
| SGLT2 inhibitors               | **Empagliflozin (63): reduces liver fat**                               | Volume depletion Genito-urinary infections                                                       | Inadequate evidence                                                                               |
| Ursodeoxycholic acid           | • Has shown reduction in transaminases without a histological improvement|                                                                                                  | NOT recommended for treatment of NAFLD (63)                                                      |
| Omega-3 fatty acids            | • Have not shown significant effects on transaminases or liver histology |                                                                                                  | NOT recommended for treatment of NAFLD (64,65,66).                                             |
| Statins                        |                                                                         |                                                                                                  | Not recommended for the treatment of NAFLD but can be safely used to treat associated dyslipidemia |
| **Ongoing Phase III trials**   |                                                                         |                                                                                                  |                                                                                                   |
| Obeticholic acid (OCA) (REGENERATE) | • Has shown promise in improve liver histology (NAFLD activity score) without worsening fibrosis |                                                                                                  | Further studies on clinical outcomes are awaited (67)                                           |
| Elafibranor (RESOLVE-IT) and Cencrineroc (CENTAUR) | • Shown promise by improving liver histology (NAFLD activity score) without worsening fibrosis |                                                                                                  | Further studies on clinical outcomes are awaited                                                  |
Management of risk factors and associations

Management of type 2 diabetes mellitus, dyslipidemia and metabolic syndrome according to available guidelines is of paramount importance to reduce the associated cardiovascular risk. Apart from the lipid lowering effects and cardiovascular risk reduction, statins have shown benefits in improving liver histology including fibrosis (68). Statins are safe to be used in patients with less than 3 fold elevation of transaminases, however routine prescription is not recommended in patients with decompensated cirrhosis and acute liver failure(69)(70). In addition to well established cardiovascular disease prevention, aspirin has shown to reduce NAFLD related fibrosis (71).

Liver Transplantation

NASH poses unique challenges in liver transplantation. NASH-related cirrhosis is the fastest growing indication for liver transplantation worldwide (72). At present, NASH related cirrhosis is the most common indication for liver transplant in Sri Lanka (73). Deceased and live donor grafts will be limited by the increasing prevalence of NAFLD in the general population(74).When compared to non-NASH cirrhosis liver transplant recipients, NASH cirrhosis recipients show similar survival rates at 1, 3, and 5 years. However, they are more likely to die from cardiovascular diseases or sepsis(75). Recurrence of NASH in the graft following liver transplant is not uncommon(76).

Pre-transplant considerations

A transplant recipient with NASH tends to be older, with higher BMI and metabolic comorbidities(77). Obesity is strongly associated with sarcopenia, which is an independent predictor of post-transplant mortality and graft loss(78). When corrected for ascites, higher BMI does not appear to independently confer an increased risk of mortality or allograft failure(79). Considering the high cardiovascular risk, non-invasive and invasive cardiac assessment is recommended as appropriate (79). Both statins and aspirin could be safely used in patients with decompensated cirrhosis and coronary artery disease undergoing liver transplant evaluation (80). Outcomes of NASH-related or cryptogenic cirrhosis appear to be similar to that of alcohol-related cirrhosis without liver transplantation (81).

Post-transplant considerations

Immunosuppressive protocols which include short term and low dose steroids and calcineurin-inhibitors are preferred (77). NASH cirrhosis liver transplant recipients are at high risk of metabolic syndrome, recurrent/de novo NAFLD and cardiovascular diseases in comparison to general population(82). All transplant recipients should be counselled to continue adherence to the lifestyle modifications.

Recurrent and de novo NAFLD

De novo (20-40%) and recurrent (30-100%) graft steatosis after liver transplant is common(83). Recurrent disease may present more frequently and may progress more rapidly than de novo disease, but the development of allograft NASH cirrhosis is rare (<2% at 10 year follow up)(84). Management is similar to native NAFLD(83).

Donor Hepatic steatosis

When persons with NAFLD are considered for liver allograft donation, severely (>60 %) steatotic grafts are associated with increased risk of poor graft function, whilst moderate-severe (>30 %) steatotic grafts are associated with reduced graft survival(85).

Follow up The optimal follow-up of patients with NAFLD is yet to be determined. Therefore, follow up should be individualized considering the risk of progression, and underlying metabolic conditions. Monitoring should include routine biochemistry (ALT, AST), assessment of co-morbidities, complications and non-invasive monitoring of fibrosis.

Follow up should be every three to six months if major therapeutic changes are done. Six-monthly for patients with advanced fibrosis. Six to twelve monthly, if stable on therapy. Yearly, if young and having low risk of fibrosis. Patients with NASH-related cirrhosis should be offered standard care for cirrhosis in addition to management of comorbidities.

Key recommendations

1. Establish the diagnosis of NAFLD
Ultrasound, hepatic panel, non-invasive markers of liver disease severity

Rule out other causes of fatty liver

2. Establish metabolic syndrome and cardiovascular (CV) risk
Obesity, Hypertension, T2DM, Dyslipidemia, CV risk factors

3. Assess lifestyle and co-morbidities

Daily activity, exercise, dietary history, alcohol and smoking habits, medical co-morbidities and current medications

4. Staging of NAFLD/ NASH/ Fibrosis severity

Non-invasive markers of fibrosis (FIB-4, NFS, transient elastography)

Liver biopsy to exclude secondary fatty liver, fibrosis staging in high risk

5. Therapeutic approaches via a MDT

Weight loss > 5-10% of body weight (dietary and exercise counselling)

Pharmacological modification for each metabolic syndrome component

Liver directed therapies for NASH or established fibrosis

Bariatric intervention in selected patients Appropriate management of NASH-related cirrhosis

6. Follow up

3-6 months if major therapeutic changes are done. 6-monthly for patient with advanced fibrosis. 6-12 monthly if stable on therapy. Yearly if young and low risk of fibrosis.

List of abbreviations

| Abbreviation | Definition |
|--------------|------------|
| ALP          | Alkaline Phosphatase |
| ALT          | Alanine Aminotransferase |
| AST          | Aspartate Aminotransferase |
| BAT          | Brown adipose tissue |
| BMI          | Body Mass Index |
| CVD          | Cardiovascular disease |
| DNL          | De novo lipogenesis |
| ESLD         | End Stage Liver Disease |
| FC           | Free cholesterol |
| FFA          | Free fatty acids |
| FIB-4        | Fibrosis-4 |
| GLP-1        | Glucagon like peptide 1 |
| GNG          | Gluconeogenesis |
| HCC          | Hepatocellular carcinoma |
| HS           | Hepatic steatosis |
| HDL          | High density lipoproteins |
| IR           | Insulin resistance |
| IHD          | Ischaemic heart disease |
| LDL          | Low density lipoprotein |
| MBOAT7       | Membrane bound O-acetyltransferase domain containing 7 |
| MDT          | Multidisciplinary team |
| NAFL         | Nonalcoholic fatty liver |
| NAFLD        | Non-alcoholic fatty liver disease |
| NAS          | NAFLD Activity Score |
| NASH         | Nonalcoholic steatohepatitis |
| NFS          | NASH fibrosis score |
| PNPLA3       | Patatin-like phospholipase domain containing protein 3 |
| PPARY        | Peroxisome proliferator activated receptor gamma |
Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. Hepatology. 2018;67(1):328–57.

2. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64(1):73–84.

3. VanWagner LB, Armstrong MJ. Lean NAFLD: A not so benign condition? Hepatol Commun. 2018;2(1):5–8.

4. Kumar R, Mohan S. Non-alcoholic Fatty Liver Disease in Lean Subjects: Characteristics and Implications. J Clin Transl Hepatol. 2017;5:216–23.

5. Herath HMM, Kodikara I, Weeraratna TP, Liyanage G. Prevalence and associations of non-alcoholic fatty liver disease (NAFLD) in Sri Lankan patients with type 2 diabetes: A single center study. Diabetes Metab Syndr Clin Res Rev [Internet]. 2019;13(1):246–50. Available from: https://doi.org/10.1016/j.dsx.2018.09.002

6. Dassanayake AS, Kasturiratne A, Rajindrajith S, Kalubowila U, Chakrawarthis S, De Silva AP, et al. Prevalence and risk factors for non-alcoholic fatty liver disease among adults in an urban Sri Lankan population. J Gastroenterol Hepatol. 2009;24(7):1284–8.

7. Pinidiyapathirage M, Dassanayake AS, Rajindrajith S, Kalubowila U, Kato N, Wickremasinghe A, et al. Non-alcoholic fatty liver disease in a rural, physically active, low income population in Sri Lanka. BMC Res Notes. 2011;4:0–4.

8. Niriella MA, Pathmeswaran A, De Silva ST, Kasturiratna A, Perera KR, Subasinghe SKCE, Kodisinghe SK, Piyaratna TACL, Vithiya K, Dassanayaka AS, De Silva AP, Wickremasinghe AR, Takeuchi F, Kato N, de Silva HJ. Incidence and risk factors for non-alcoholic fatty liver disease among adults: a 7-year follow-up study in an urban Sri Lankan community. Liver International 2017; 37(11):1715–22.

9. Dowman JK, Tomlinson JW, Newsome PN. Review Pathogenesis of non-alcoholic fatty liver disease. 2010;(November 2009):71–83.

10. Petta S, Gastaldelli A, Rebello E, Bugianesi E, Messa P, Miele L, et al. Pathophysiology of Non Alcoholic Fatty Liver Disease. 2016;

11. Seko Y, Yamaguchi K, Itoh Y. The genetic backgrounds in nonalcoholic fatty liver disease. Clin J Gastroenterol [Internet]. 2018;11(2):97–102. Available from: https://doi.org/10.1007/s12328-018-0841-9

12. Malekzadeh R, Calich AL, Bonf E. Anti-ribosomal P protein antibody and prognosis in autoimmune hepatitisAssociation of genetic variants with non-alcoholic fatty liver disease in an urban Sri Lankan community. 2015;(2):676–9.
13. Li I, Liu D, Yan H, Wang Z, Zhao S, Wang B. Obesity is an independent risk factor for non-alcoholic fatty liver disease: evidence from a meta-analysis of 21 cohort studies. 2016;(June):510–9.

14. Lu F, Hu E, Xu L, Chen I, Wu J, Li H, et al. The relationship between obesity and the severity of non-alcoholic fatty liver disease: systematic review and meta-analysis. 2018;4124.

15. Nirriella MA, Kasturiratne A, Pathmeswaran A, De Silva ST, Perera KR, Subasinghe SKCF, et al. Lean non-alcoholic fatty liver disease (lean NAFLD): characteristics, metabolic outcomes and risk factors from a 7-year prospective, community cohort study from Sri Lanka. Hepatol Int [Internet]. 2018;(0123456789). Available from: https://doi.org/10.1007/s12072-018-9916-4

16. Amiri N, Atan D, Koushki M, Motedayen M, Dousti M, Sayehmiri F. Type 2 diabetes mellitus and non-alcoholic fatty liver disease: a systematic review and meta-analysis. 2017;(1):1–7.

17. Mantovani A, Byrne CD, Bonora E. Nonalcoholic Fatty Liver Disease and Risk of Incident Type 2 Diabetes: A Meta-analysis. 2018;41(February):372–82.

18. Wu K, Kuo P, Su S, Chen Y, Yeh M, Huang C, et al. Nonalcoholic fatty liver disease severity is associated with the ratios of total cholesterol and triglycerides to high-density lipoprotein cholesterol. J Clin Lipidol [Internet]. 2016;(100). Available from: http://dx.doi.org/10.1016/j.jacl.2015.12.026

19. Kim G, Lee HC, Choe J, Shim JH, Kim KM, Lim Y. Association between non-alcoholic fatty liver disease and cancer incidence rate. J Hepatol [Internet]. 2017; Available from: https://doi.org/10.1016/j.jhep.2017.09.012

20. Musso G, Gambino R, Tabibian JH, Ekstedt M, Kechagias S, Yoon SK, et al. Association of Non-alcoholic Fatty Liver Disease with Chronic Kidney Disease : A Systematic Review and. 2014;11(7).

21. Younossi ZM. The Epidemiology of Nonalcoholic Steatohepatitis. 2018;11(4):92–4.

22. Nasr P, Ignatova S, Kechagias S, Ekstedt M. Natural History of Nonalcoholic Fatty. 2018;2(2):199–210.

23. Rafiq N, Bai C, Fang YUN, Strshold M, McCullough A, Gramlich T, et al. Long-Term Follow-Up of Patients With Nonalcoholic Fatty Liver. YJCGH [Internet]. 2009;7(2):234–8. Available from: http://dx.doi.org/10.1016/j.jcah.2008.11.005

24. Ong JP, Pitts A, Younossi ZM. Increased overall mortality and liver-related mortality in non-alcoholic fatty liver disease q. 2008;49:608–12.

25. Younossi ZM, Goodman Z, Stepanova M, Rafiq N, Henry L, Loomba R, et al. Nonalcoholic Steatofibrosis. 2017;1(5):421–8.

26. Kechagias S, Nasr P, Fredrikson M, Sta P. Fibrosis Stage Is the Strongest Predictor for Disease-Specific Mortality in NAFLD After Up to 33 Years of Follow-Up . 2014;1–8.

27. Hagström H, Nasr P, Ekstedt M, Hammar U, Stål P, Hultcrantz R, et al. Fibrosis stage but not NASH predicts mortality and time to development of severe liver disease in biopsy-proven NAFLD. J Hepatol. 2017;67(6):1265–73.

28. Marchesini G, Roden M, Vettor R. Response to: Comment to “EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease.” J Hepatol [Internet]. 2017;66(2):466–7. Available from: http://dx.doi.org/10.1016/j.jhep.2015.11.004

29. Kim D, Kim WR, Kim D, Kim WR. Non-Obese Fatty Liver Disease. Clin Gastroenterol Hepatol [Internet]. 2016; Available from: http://dx.doi.org/10.1016/j.cgh.2016.08.028

30. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. Hepatology [Internet]. 2018;67(1):328–57. Available from: http://doi.wiley.com/10.1002/hep.29367

31. Esterson YB. Radiological Imaging in Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis. Clin Liver Dis [Internet]. 2017; Available from: https://doi.org/10.1016/j.cld.2017.08.005

32. Report of a WHO Expert Consultation. Waist Circumference and Waist-Hip Ratio Report of a WHO Expert Consultation. 2008;(December):8–11.
33. Dyson JK, Anstee QM, Mcpherson S. Non-alcoholic fatty liver disease: a practical approach to diagnosis and staging. 2014;211–8.

34. Kaswala DH, Lai M, Afdhal NH. Fibrosis Assessment in Nonalcoholic Fatty Liver Disease (NAFLD) in 2016. Dig Dis Sci. 2016;

35. Vilar-Gomez E, Martinez-Perez Y, Calzadilla-Bertot L, Torres-Gonzalez A, Gra-Oramas B, Gonzalez-Fabian L, et al. Weight loss through lifestyle modification significantly reduces features of nonalcoholic steatohepatitis. Gastroenterology [Internet]. 2015;149(2):367-378.e5. Available from: http://dx.doi.org/10.1053/j.gastro.2015.04.005

36. Kittichai Promrat, David E Kleiner, Heather M Niemeier, Elizabeth Jackvony, Marie Kearns, Jack R Wands, Joseph Fava and R R W. Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis (NASH). Hepatology. 2010;1(51):121–9.

37. Harrison SA, Fecht W, Brunt EM, Neuschwander-tetri BA. Orlistat for Overweight Subjects with Nonalcoholic.

38. Wong VW, Chan RS, Wong GL, Cheung H, Chu WC, Yeung DK, et al. Community-based lifestyle modification programme for non-alcoholic fatty liver disease: a randomized controlled trial. J Hepatol [Internet]. 2013; Available from: http://dx.doi.org/10.1016/j.jhep.2013.04.013

39. Utz W, Haas V, Hermansdorf M, Haufe S, Engeli S, Kast P, et al. Randomized Comparison of Reduced Fat and Reduced Carbohydrate Hypocaloric Diets on Intrahepatic Fat in Overweight and Obese Human Subjects. 2011;1504–14.

40. Abdelmalek MF, Suzuki A, Guy C, Unalp-arida A, Colvin R, Johnson RJ. Increased Fructose Consumption Is Associated with Fibrosis Severity in Patients with Nonalcoholic Fatty Liver Disease. 2010;1961–71.

41. Zelber-sagi S, Ratziu V, Oren R. Nutrition and physical activity in NAFLD: An overview of the epidemiological evidence. 2011;17(29):3377–89.

42. Bill F, Foundation MG. Articles Alcohol use and burden for 195 countries and territories , 1990 – 2016 : a systematic analysis for the Global Burden of Disease Study 2016. 2018;1015–35.

43. Heath RD, Brahmbhatt M, Tahan AC, Ibdah JA, Tahan V, Heath RD, et al. Coffee: The magical bean for liver diseases. 2017;9(15):689–96.

44. Kantarzis K, Thamer C, Peter A, Machann J, Schick F, Schraml C, et al. High cardiorespiratory fitness is an independent predictor of the reduction in liver fat during a lifestyle intervention in non-alcoholic fatty liver disease. 2009;1281–8.

45. Nagamatsu T. Impact of physical activity on nonalcoholic steatohepatitis in people with nonalcoholic simple fatty liver: A prospective cohort study. Prev Med (Baltim) [Internet]. 2016; Available from: http://dx.doi.org/10.1016/j.ypmed.2016.04.020

46. Keating SE, Hackett DA, Parker HM, Connor HTO, Gerofi JA, Sainsbury A, et al. Effect of aerobic exercise training dose on liver fat and visceral adiposity. J Hepatol [Internet]. 2015;xxx. Available from: http://dx.doi.org/10.1016/j.jhep.2015.02.022

47. Trial R, Bacchi E, Negri G, Targher G, Lanza M, Zoppini G, et al. Both Resistance Training and Aerobic Training Reduce Hepatic Fat Content in Type 2 Diabetic Subjects With Nonalcoholic Fatty Liver Disease (the RAED2 Randomized Trial). 2013;1287–95.

48. Laursen TL, Hagemann CA, Wei C, Kazankov K, Thomsen KL, Knop FK, et al. Bariatric surgery in patients with non-alcoholic fatty liver disease - from pathophysiology to clinical effects. 2019;11(2):138–49.

49. Garvey WT, Mechanick JI. / American Association of Clinical Endocrinologists and American College of Endocrinology Comprehensive Clinical Practice Guidelines for Medical Care of Patients with Obesity. 2016;22(July).

50. Lassailly G, Caiazzo R, Buob D, Pigeyre M, Verkindt H, Lahreuche J, et al. Bariatric Surgery Reduces Features of Non-alcoholic Steatohepatitis in Morbidly Obese Patients. Gastroenterology [Internet]. 2015; Available from: http://dx.doi.org/10.1053/j.gastro.2015.04.01
51. Udai Wijetunga, Uditha Bulugahapitiya, Thejana Wijeratne, Anuradha Jayasuriya, Gowri Ratnayake, Vidumini Kaluarachchi, Sonali Gunatilake, Charini Silva AG. SAT-104 Reversal of Nonalcoholic Fatty Liver Disease with Bariatric Surgery in South Asians: Has the Cure Been Finally Found? Real World Data From a Sri Lankan Tertiary Care Setting. J Endocr Soc. Volume 3(Issue Supplement_1).

52. Udai Wijetunga, Uditha Bulugahapitiya, Thejana Wijeratne, Anuradha Jayasuriya, Gowri Ratnayake, Vidumini Kaluarachchi, Sonali Gunatilake, Charini Silva AG. SAT-111 Reversal of Nonalcoholic Fatty Liver Disease with Bariatric Surgery in South Asians: Does the Type of Surgery Matter? J Endocr Soc. 2019;Volume 3(Issue Supplement_1).

53. Mathurin P, Hollebecque A, Armalsteen L, Buob D, Leteurtre E, Caiazzo R, et al. CLINICAL ADVANCES IN LIVER, PANCREAS, AND BILIARY TRACT Prospective Study of the Long-Term Effects of Bariatric Surgery on Liver Injury in Patients Without Advanced Disease. YGAST [Internet]. 2009;137(2):532–40. Available from: http://dx.doi.org/10.1053/j.gastro.2009.04.052

54. Mosko JD, Nguyen GC. Increased Perioperative Mortality Following Bariatric Surgery Among Patients With Cirrhosis. YJCGH [Internet]. 2011;9(10):897–901. Available from: http://dx.doi.org/10.1016/j.jchb.2011.07.007

55. Dulai PS, Singh S, Patel J, Soni M, Prokop LJ, Younossi Z, et al. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: Systematic review and meta-analysis. Hepatology. 2017;65(5):1557–65.

56. Sanyal AJ, Chalasani N, Kowdley K V, McCullough A, Diehl AM, Bass NM, et al. Pioglitazone, Vitamin E, or Placebo for Nonalcoholic Steatohepatitis. N Engl J Med. 2010 May;362(18):1675–85.

57. Sato K, Gosho M, Yamamoto T, Kobayashi Y, Ishii N, Ohashi T, et al. Vitamin E has a beneficial effect on nonalcoholic fatty liver disease: a meta-analysis of randomized controlled trials. Nutrition. 2015;31(7–8):923–30.

58. McCullough A, Diehl AM, Bass NM, Ph D, Clark J, Brunt EM, et al. Pioglitazone, Vitamin E, or Placebo for Nonalcoholic Steatohepatitis. 2019;1675–85.

59. LI Y, LIU L, WANG B, WANG J, CHEN D. Metformin in non-alcoholic fatty liver disease: A systematic review and meta-analysis. Biomed Reports. 2012;1(1):57–64.

60. Tacelli M, Celsa C, Magro B, Giannetti A, Pennis G, Spatola F, et al. Antidiabetic Drugs in NAFLD: The Accomplishment of Two Goals at Once? Pharmaceuticals. 2018;11(4):121.

61. Donadon V, Balbi M, Mas MD, Casarin P, Zanette G. Metformin and reduced risk of hepatocellular carcinoma in diabetic patients with chronic liver disease. Liver Int. 2010;30(5):750–8.

62. Armstrong MJ, Gaunt P, Aithal GP, Barton D, Hull D, Parker R, et al. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis ( LEAN ): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. 1:679–90.

63. Kuchay MS, Krishan S, Mishra SK, Farooqui KJ, Singh MK, Wasir JS, et al. Effect of Empagliflozin on Liver Fat in Patients With Type 2 Diabetes and Nonalcoholic Fatty Liver Disease: A Randomized Controlled Trial (E-LIFT Trial). Diabetes Care [Internet]. 2018 Aug 1;41(8):1801 LP – 1808. Available from: http://care.diabetesjournals.org/content/41/8/1801.abstract

64. Argo CK, Patrie JT, Lackner C, Henry TD, de Lange EE, Weltman AL, et al. Effects of n-3 fish oil on metabolic and histological parameters in NASH: a double-blind, randomized, placebo-controlled trial. J Hepatol. 2013 Jan;62(1):190–7.

65. Sanyal AJ, Abdelmalek MF, Suzuki A, Cummings OW, Chojkier M. No significant effects of ethyl-eicosapentanoic acid on histologic features of nonalcoholic steatohepatitis in a phase 2 trial. Gastroenterology. 2014 Aug;147(2):377–84.e1.

66. Scortetti E, Bhata I, McCormick KG, Clough GF, Nash K, Hodson I, et al. Effects of purified eicosapentaenoic and docosahexaenoic acids in nonalcoholic fatty liver disease: results from the Welcome* study. Hepatology. 2014 Oct;60(4):1211–21.

67. Neuschwander-tetri BA, Loomba R, Sanyal AJ, Lavine JE, Natta MI. Van, Abdelmalek MF, et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. Lancet [Internet]. 2014;6736(14):1–11. Available from: http://dx.doi.org/10.1016/S0140-6736(14)61933-4
68. Stahl EP, Dhindsa DS, Lee SK, Sandesara PB, Chalasani NP, Sperling LS. Nonalcoholic Fatty Liver Disease and the Heart: JACC State-of-the-Art Review. J Am Coll Cardiol. 2019;73(8):948–63.

69. Leoni S, Tovoli F, Napoli L, Serio I, Ferri S, Bolondi L. Current guidelines for the management of non-alcoholic fatty liver disease: A systematic review with comparative analysis. World J Gastroenterol. 2018;24(30):3361–73.

70. Sigler MA, Congdon L, Edwards KL. An Evidence-Based Review of Statin Use in Patients With Nonalcoholic Fatty Liver Disease. Clin Med Insights Gastroenterol. 2018;11.

71. Simon TG, Henson J, Osganian S, Masia R, Chan AT, Chung RT, et al. Daily aspirin use associated with reduced risk for fibrosis progression in patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol [Internet]. 2019;(June). Available from: https://doi.org/10.1016/j.cgh.2019.04.061

72. Goldberg D, Ditah IC, Saeian K, Lalehzari M, Aronsohn A, Gorospe EC, et al. Changes in the Prevalence of Hepatitis C Virus Infection, Nonalcoholic Steatohepatitis, and Alcoholic Liver Disease Among Patients With Cirrhosis or Liver Failure on the Waitlist for Liver Transplantation. Gastroenterology [Internet]. 2017;152(5):1090-1099.e1. Available from: http://dx.doi.org/10.1016/j.gastro.2017.01.003

73. Report S. Cryptogenic cirrhosis is the leading cause for listing for liver transplantation in Sri Lanka. 2013;32(December):397–9.

74. Silva H, Siriwardana RC, Niriella MA, Dassanayake AS, Liayange CA, Gunathilake B, De Silva HJ. Nonalcoholic fatty liver disease among potential live liver donors--a preliminary experience from Sri Lanka. Indian J Gastroenterol. 2014 Nov;33(6):573-4.

75. Wang X, Li J, Riaz DR, Shi G, Liu C, Dai Y. Outcomes of liver transplantation for nonalcoholic steatohepatitis: A systematic review and meta-analysis. Clin Gastroenterol Hepatol [Internet]. 2014;12(3):394-402.e1. Available from: http://dx.doi.org/10.1016/j.cgh.2013.09.023

76. Siriwardana RC, Niriella MA, Dassanayake AS. Recurrence of graft steatosis after liver transplantation for cryptogenic cirrhosis in recently commenced liver transplant program. Indian J Gastroenterol [Internet]. 2016;1–3. Available from: http://dx.doi.org/10.1007/s12664-016-0653-0

77. Harmee Malhi, Alina M. Allen, and Kymberly D. WattRiaz N, Wolden SL, Gelblum DY, Eric J. Nonalcoholic fatty liver: Optimizing pre-transplant selection and post-transplant care to maximize survival. Curr Opin Organ Transpl. 2016;2(21):99–106.

78. Tandon P, Ney M, Irwin I, Ma MM, Gramlich L, Bain VG, et al. Severe muscle depletion in patients on the liver transplant wait list: Its prevalence and independent prognostic value. Liver Transplant. 2012;18(10):1209–16.

79. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rindell M, et al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. Hepatology. 2018;67(1):328–57.

80. Patel SS, Guzman LA, Lin FP, Pence T, Reichman T, John B, et al. Utilization of aspirin and statin in management of coronary artery disease in patients with cirrhosis undergoing liver transplant evaluation. Liver Transplant. 2018;24(7):872–80.

81. Senanayake SM, Niriella MA, Weerasinghe SK, Kasturiratne A, Alwis JP De, Silva AP De, et al. Survival of patients with alcoholic and cryptogenic cirrhosis without liver transplantation: a single center retrospective study. BMC Res Notes. 2012 Dec 25; 663.

82. Gitto S, Marra F, De Maria N, Bihi F, Villa E, Andreone P, et al. Nonalcoholic steatohepatitis before and after liver transplant: keeping up with the times. Expert Rev Gastroenterol Hepatol [Internet]. 2019;13(2):173–8. Available from: https://doi.org/10.1080/17474124.2019.1551132

83. Germani G, Laryea M, Rubbia-Brandt L, Egawa H, Burra P, O’Grady J, et al. Management of Recurrent and de Novo NAFLD/NASH after Liver Transplantation. Transplantation. 2019;103(1):57–67.

84. Narayanan P, Mara K, Izzy M, Dlerkhising R, Heimbach J, Allen AM, et al. Recurrent or de Novo Allograft Steatosis and Long-term Outcomes after Liver Transplantation. Transplantation. 2019;103(1):E14–21.

85. Chu MJJ, Dare AJ, Phillips ARJ, Bartlett ASJ.R. Donor Hepatic Steatosis and Outcome After Liver Transplantation: a Systematic Review. J Gastrointest Surg. 2015;19(9):1713–24.
The Colombo Declaration, 2019

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Preamble

While diabetes is usually described as an epidemic, it has actually gained endemic status in many parts of the world. Data from the International Diabetes Federation (IDF) suggest a current (2017) global prevalence of 425 million; with the number expected to increase to 629 million by 2040 (1,2). There are 78 million people living with diabetes in South Asia today. This number is expected to double to reach 140 million by 2040.

A healthy diet, regular physical activity, and maintaining a normal body weight are the three key strategies to prevent or delay the onset of type 2 diabetes (T2D). Diabetes can be treated and its consequences avoided or delayed with healthy eating practices, physical activity, medication and regular screening and treatment for complications.

The impact of diabetes on individual, family and societal health is enormous. First of all, economic productivity is impaired, as two-thirds of persons affected with diabetes are of working age. Diabetes is also a major cause of blindness, renal failure, heart attack, stroke and lower limb amputation. In 2016, an estimated 1.6 million deaths were directly caused by diabetes. Another 2.2 million deaths were attributed to high blood glucose according to 2012 statistics (2). Every year, 1.2 million lives in South Asia are lost prematurely to diabetes. At a global level, diabetes care consumes 12% of the total health expenditure. Therefore, it is imperative to prevent and control diabetes, and to do so, with immediate effect.

Levels of prevention

Prevention of disease can be carried out at various levels. Conventionally, these are classified as primary, secondary and tertiary (3). In recent years, the concepts ‘primordial prevention’ and ‘quaternary prevention’ have also gained attention (4,5). To this list, we propose the addition of another level, which we term ‘quinary prevention’. The definitions of the traditional levels of prevention are given in the table below.
The novel concept of quaternary prevention calls for the avoidance of overdiagnosis, over-labeling, and overtreatment (5). This much-needed cautionary wisdom reminds medical professionals to refrain from unnecessary investigations and treatment, while ensuring that appropriate care is provided to all.

Modern technology has facilitated the spread of information through diverse communication channels. While such media is useful in conveying health-related messages, they can also become the means of spreading misinformation. This is termed e-hearsay. Put simply, quinary prevention is the “means of preventing health-related hearsay or misinformation, or its ill effects on the health of individuals” (6). Thus, quinary prevention of diabetes would minimize or prevent the spread of misinformation related to diabetes.

These levels of prevention have been conceptualized and implemented by member societies of the South Asian Federation of Endocrine Societies (SAFES), through multilevel prevention programs in the South Asian region.

| Level of Prevention | Description |
|---------------------|-------------|
| Primordial prevention | Considered as a subset of primary prevention, implies the institution of preventive measures in the absence of risk factors, in the population at large. |
| Primary prevention | The institution of preventive measures in the absence of disease, in persons in whom risk factors are present |
| Secondary prevention | Measures for early detection and treatment of disease to prevent complications. |
| Tertiary prevention | Strategies put in place to minimize ill-health due to pre-existing complications, and to prevent their worsening |
| Quinary Prevention | Addressing the spread of myths and misconceptions regarding diabetes by advanced means of communication. |

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Primordial prevention

Although diabetes causation is multifactorial, interventions that target lifestyle can reduce the incidence of diabesity (7). These entail changes in personal lifestyle and environment backed by proper legislature and societal action. Some of the legislative changes that could be implemented include taxation on unhealthy foods and subsidies to promote consumption of healthy food. Additionally, intervention in schools and workplaces to encourage the consumption of healthy food and increase in physical activity would also contribute to creating a healthy society (8,9). Finally, in order to reduce fetal ill effects and to minimize adverse intrauterine developmental programming, it is imperative to encourage young women to be “healthy” in order to become “healthy mothers”. A program that screens for gestational diabetes (GDM) and educates pregnant women not only reduces the associated maternal, perinatal morbidity and mortality, but also ensures the transgenerational prevention of several chronic diseases (10).

Included below are a few examples of primordial prevention initiatives that have been successfully implemented in South Asia.
The Bangladesh Diabetes Sanity (BADAS) has introduced a nationwide preconception care program to disseminate the concept of ‘healthy mother- healthy baby’ towards mitigating the fetal origins of adult disease. A healthy lifestyle for future fathers is also important for the health outcome of children. The Endocrine Associations of Bangladesh, Nepal, Pakistan and Afghanistan regularly conduct public awareness campaigns to encourage a healthy lifestyle. The Diabetes Education Foundation (DEF) based in India has been conducting public awareness programs regularly, for almost two decades, and has pioneered the launch of health and nutrition education initiatives among school children to spread awareness of obesity amongst the youth. The Sri Lankan government has included multiple legislations that include sugar tax, color coded labelling of beverages and packaged food, warning messages on cigarette packages and a ban on advertising of alcohol. In addition, the Sri Lanka College of Endocrinologists (SLCE) has launched the Sri Lanka Diabetes and Cardiovascular Disease Initiative (SLDC) that works with communities on health promotion and in the training of health sector staff to screen and educate all pregnant women on the ‘healthy mother- healthy baby’ concept. The SAFES has conceptualized and launched the Run For Life® campaign that encourages school children to engage in regular sports activity, and the Pakistan Endocrine Society has spearheaded such campaigns in schools across the country. This campaign received international recognition from the International Society of Endocrinology for the concept and its execution.

Although civil society and grassroots movements are crucial in this effort, the prevention and management of diabetes also requires the highest political attention. To this end, the SAFES and its member societies are working together with the International Society of Endocrinology (11) to ensure that diabetes remains a priority on the global NCD agenda of the WHO while more prevention efforts are undertaken by governments to halt the epidemic, especially in Lower Middle-Income Countries. The Year 2013 was an important year for diabetes (and NCDs) as WHO adopted voluntary NCD targets. An overall goal of a 25% reduction in premature mortality from NCD by 2025 and within the nine voluntary targets on NCDs including a 0% increase in diabetes and obesity prevalence, and an 80% access to essential medicines and devices by 2025 was included (12). However, it is regrettable that despite global political commitment, many countries have not made adequate progress to meet their targets.

Primary prevention

Multiple trials have shown that the prevention of diabetes is possible in those who are obese or overweight and in those who have impaired glucose tolerance (13). The key interventions that have been shown to be effective include vigorous physical activity and adoption of a lower calorie diet that is rich in fiber and low in carbohydrates. Weight reduction is yet another powerful tool for the prevention of diabetes in those at risk. The identification of high-risk groups such as prediabetes, gestational diabetes mellitus (GDM) and obesity is crucial to the success of primary prevention. Studies from India, Pakistan and Bangladesh have shown the benefits of targeting women with a history of GDM to prevent diabetes. The Dhaka Declaration of 2015, released by SAFES, underscored the importance of this high-risk cohort; in choosing GDM as its theme. Guidelines for the Management of GDM were published in the Journal of Pakistan Medical Association and endorsed by The South Asian Federation of Obstetrics and Gynecology (SAFOG).

The Indian Diabetes Prevention Program used lifestyle modification and metformin to prevent diabetes in a high-risk cohort of prediabetes. Telephonic SMS (short message service) based contact has also been demonstrated to reduce the risk of diabetes among the Indian population. BADAS and SLDC have conducted programs using multiple integrated interventions such as mass awareness, education and capacity building, screening and early intervention of risk factors, creating ambassadors, mobilizing community leaders, use of folk songs, utilizing religious leaders and school teachers, awareness programs in transport media and a multimedia campaign. A comprehensive primary prevention program through the institution of screening and early treatment at primary health care settings has been implemented in Bangladesh and Sri Lanka.

Secondary prevention

Timely diagnosis of diabetes allows the early institution of both non-pharmacological and pharmacological therapy. Studies of semiparental value, such as the Diabetes Control and Complications Trial (DCCT) in type 1 diabetes, and The United Kingdom Prospective Diabetes Study (UKPDS) in T2D, have proven the effectiveness of glycemic control in the reduction of chronic complications. The Steno-2 study, which evaluated a multifactorial intensive treatment approach in T2D and microalbuminuria, achieved a 7.9 year increase in survival over a 21-year follow up period. These data, along with encouraging results from various cardiovascular outcome
trials, emphasize the importance of secondary prevention in diabetes (14).

Modern endocrinology seeks to provide timely, comprehensive vasculo-metabolic care, in a patient-centered manner, to all persons with diabetes. Diabetes has a heavy disease burden on the healthcare system and South Asian endocrinologists are tasked with educating their peers on the advances in the secondary prevention of diabetes.

**Tertiary prevention**

The philosophy of prevention holds true for all people, irrespective of their diabetic status, or the presence of complications. In a tertiary prevention mode, treating physicians aim to minimize the impact of chronic complications. Post-myocardial infarction rehabilitation, renal replacement therapy, and corrective intervention for diabetic retinopathy are examples of tertiary prevention. Modern diabetes therapies allow the slowing or regression of atherosclerosis and chronic renal disease as well as the prevention of heart failure in high-risk patients. Patients with foot complications can be provided rehabilitation and spared from amputation through multidisciplinary intervention. A close collaboration between endocrine care providers and colleagues from other specialties is required to ensure appropriate tertiary prevention and care of diabetes. The International Federation of Diabetes (IDF) has also established a foot care center and research in the region. The Pakistan Endocrine Society hosts foot clinics and guides and works with other regional countries. The Indian flagship National Health Protection scheme (15) aims at making interventions in primary, secondary and tertiary care systems, covering both preventive and promotive health, to address healthcare holistically. The Sri Lankan and Maldivian national health care systems provide access to primary, secondary and tertiary care free of charge and have made several structural modifications of delivery systems to face the challenge of NCDs.

**Quinary prevention**

As diabetes care becomes more and more complex, the need to practice quaternary prevention has become more urgent. Endocrinologists should thus ensure that evidence-based methods of screening, diagnosing and managing diabetes are followed. Improper diagnosis of diabetes and its subtypes such as pancreatic or gestational diabetes may lead to inappropriate management strategies and suboptimal outcomes. Moreover, the unwarranted use of ‘labels’ such as GDM may have psychosocial ramifications or may result in marital disharmony and domestic discord. This phenomenon is commonly encountered in certain gender-sensitive cultures of South Asia (16). Overtreatment using novel glucose-lowering drugs, which prescribers are less familiar with, may be associated with avoidable adverse events. Such therapeutic mishaps should be avoided at all costs.

Regular continuing medical education (CME) programs conducted by qualified endocrinologists are needed to instill the philosophy of quaternary prevention in physicians who deal with diabetes. SAFES and its member organizations contribute to the cause of quaternary prevention by organizing biennial South Asian summits, national endocrine conferences, and other CMEs of high academic standard standing. The Journal of Pakistan Medical Association has carried published a series of articles on the quaternary prevention of endocrine conditions, including diabetes (17). The SLDC has trained more than 15,000 health sector workers on the prevention and treatment of diabetes. SAFES has partnered with the Public Health Foundation of India (18) to provide training courses i.e. Certificate Course in Evidence-based Diabetes Mellitus (CCEBDM), Certificate Course in GDM (CCGDM) and Certificate Course in Thyroid Disorders (CCMID) to more than 4000 doctors. BADAS has offered distance-learning certificate program to 16,000 primary care doctors.

**Quinary prevention**

Hearsay, including e-hearsay, has emerged as a significant barrier to diabetes care. The spread of myths and misconceptions regarding diabetes has been fueled by advanced means of communication. A concerted and sustained campaign led by the endocrine fraternity is necessary to educate people living with diabetes, and the society at large, by providing them the right knowledge and information about the pathogenesis, management and prevention of diabetes. With the power of multimedia at their disposal, each citizen has become a broadcaster; it is in harnessing this power to propagate the right messages that major societal changes can be achieved. Indian endocrinologists use WhatsApp and Twitter to direct the public towards authentic centers for diabetes care. SLDC and BADAS have used a systematic multimedia campaign to educate and provide information. Bangladesh has utilized the services of religious leaders to spread awareness about GDM and its prevention.

**Resolution**

We, the participants of SLENDO 2019; the Sri Lanka College of Endocrinologists (SLCE)/ the South Asian Federation of Endocrine Societies (SAFES)
International Society of Endocrinology (ISE) conference, meeting in Colombo from 1st to 3rd August 2019 hereby resolve to:

- Promote the concept of prevention of diabetes, at all levels, ranging from primordial to quinary.
- Practice preventive strategies as an integral part of diabetes and endocrine care.
- Prioritize the prevention of diabetes in public outreach and advocacy activities.
- Proactively launch activities, at micro- and meso-level, to help prevent diabetes and its complications.
- Partner with national and international societies to create and strengthen diabetes prevention programs.
- Prepare endocrine curricula and continuing education modules with an enhanced focus on the prevention of diabetes.
- Publicize best practices from Sri Lanka and the South Asian region to encourage idea-generation and sharing.

Summary

The Colombo Declaration 2019, we hope, will serve as a call for action. This Declaration will encourage:

- Communication between various stakeholders in the prevention of diabetes
- Confidence in their ability to work as an effective team
- Consolidation of existing activities and programs related to prevention of diabetes
- Conversion of existing ideas and plans into meaningful and productive action
- Creation of focused and sustained prevention plans, which will help stem the diabetes epidemic.
References

1. IDF Atlas 8th edition. Available at: https://diabetesatlas.org/. Last accessed 07 June 2019.

2. Diabetes. Available at: https://www.who.int/news-room/fact-sheets/detail/diabetes. Last accessed 07 June 2019.

3. Starfield B, Hyde J, Gérvas J, Heath I. The concept of prevention: a good idea gone astray?. *Journal of Epidemiology & Community Health*. 2008 Jul 1;62(7):580-3.

4. Zimmet PZ. Primary prevention of diabetes mellitus. *Diabetes Care*. 1988 Mar 1;11(3):258-62.

5. Jamoulle M. Quaternary prevention, an answer of family doctors to overmedicalization. *International Journal of Health Policy and Management*. 2015 Feb;4(2):61.

6. Kalra S, Kumar A. Quinary prevention. Accepted by J Pak Med Assoc

7. Kharroubi AT, Darwish HM. Diabetes mellitus: The epidemic of the century. *World Journal of Diabetes*. 2015 Jun 25;6(6):850.

8. Kalra S, Das AK, JImeno C, Nitiyanant W, Su-Yen G, Le T. The Berlin Declaration: Inspiration for primary care. J Pak Med Assoc. 2017 Dec 1;67(12).

9. Somasundaram NP, Kalupahana NS. Population-based dietary approaches for the prevention of noncommunicable diseases. *WHO South-East Asia J Public Health*. 2016;5:22-6

10. Kalra B, Kalra S, Unnikrishnan AG, Baruah MP, Khandelwal D, Gupta Y. Transgenerational karma. *Indian Journal of Endocrinology and Metabolism*. 2017 Mar;21(2):265.

11. News. Available at: https://www.isendo.org/news/. Last accessed 07 June 2019.

12. Know the NCD targets. Available at: https://www.who.int/beat-ncds/take-action/targets/en/ Last accessed 07 June 2019.

13. Schulze MB, Hu FB. Primary prevention of diabetes: what can be done and how much can be prevented?. *Annu. Rev. Public Health*. 2005 Apr 21;26:445-67.

14. Kalra S, Ved J, Baruah MP. Diabetes destiny in our hands: Achieving metabolic karma. *Indian Journal of Endocrinology and Metabolism*. 2017 May;21(3):482.

15. PM-JAY. Available at: https://www.pmjay.gov.in/ . Last accessed n 07 June 2019.

16. Kalra S, Sreedevi A, Unnikrishnan AG. Quaternary prevention and diabetes. *J Pak Med Assoc*. 2014 Nov 1;64(11):1324-6.

17. Kalra S, Gupta Y, Kalra B. Quaternary prevention and gestational diabetes mellitus. *Indian Journal of Endocrinology and Metabolism*. 2017 Jan;21(1):1.

18. Awards and recognitions. Available at: https://phfi.org/about/awards-and-recognitions/. Last accessed on 07 June 2019.
Erratum

The line "In the T2DM group, age, BMI, WC, WHR, FPG, fasting insulin and HOMA-IR correlated with serum Mg level though in the control group Mg had significant inverse correlations with BMI and fasting insulin" in the abstract of the article titled "Serum magnesium status and its correlation with insulin resistance in newly diagnosed patients with type 2 diabetes mellitus" published in volume 9, issue 1, page 11–17 (DOI: http://doi.org/10.4038/sjdem.v9i1.7367) should be replaced by "In the T2DM group, none of the age, BMI, WC, WHR, FPG, fasting insulin and HOMA-IR correlated with serum Mg level though in the control group Mg had significant inverse correlations with BMI and fasting insulin."